

Validation of Urinary Biomarkers of Hydration Status in College Athletes

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ABSTRACT

Adequate hydration is critical for optimal performance and health. Fluid requirements of collegiate athletes are unique due to training and competition, travel, school schedules, and stressors common in college environments. Inattention to these factors may contribute to suboptimal hydration. Importantly, loss of 1-2% of body weight by dehydration can impair physical and cognitive performance. As such, development of valid and reliable tools to assess hydration status in collegiate athletes is needed. The purpose of this study was to assess the validity of urine color (UC) as a measure of hydration status in collegiate athletes. A secondary purpose was to evaluate the utility of indexes of hydration status for UC and urine specific gravity (USG) established by the American College of Sports Medicine (ACSM) and the National Athletic Trainers' Association (NATA). To address this, 62 NCAA Division I collegiate athletes provided a urine sample ≤ 30 minutes of exercise for UC self-assessment (UC_{sub}) and experimenter-assessment (UC_{res}) using the UC chart developed by Armstrong et al. (1994) and for USG measurement via refractometry (1). Habitual dietary intake was assessed by 24-hr recalls. There was a significant positive correlation between USG and both UC_{sub} ($r=0.679$, $p<0.001$) and UC_{res} ($r=0.772$, $p<0.001$). In addition, the USG based on UC was inconsistent with hydration/dehydration categories established by ACSM and NATA. These findings suggest that UC, even when self-assessed by the athlete, is a valid method for assessing hydration status in NCAA division I college athletes. However, some modification of ACSM and NATA hydration categories may be warranted.

1. Armstrong LE, Maresh CM, Castellani JW, et al. Urinary indices of hydration status. *Int J Sport Nutr.* 1994;4(3):265-279. <http://www.ncbi.nlm.nih.gov/pubmed/7987361>. Accessed October 26, 2016.

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GENERAL AUDIENCE ABSTRACT

Staying well hydrated is important for athletes' health and to help them perform at their best. College athletes fluid needs are unique because of their training and competition schedules, class schedules, frequent travel, and emotional stress that is common for college students. Without conscious efforts to consume enough fluid during the day, athletes may not be well hydrated. Small decreases in body weight from dehydration can result in negative consequences for physical and mental performance. This is why it is important to develop tools that can accurately and consistently determine how hydrated athletes are. The purpose of this study was to see if urine color could accurately determine if college athletes were well hydrated or dehydrated. Also, the usefulness of hydration categories for urine color and urine specific gravity (USG) from the American College of Sports Medicine (ACSM) and the National Athletic Trainers' Association (NATA) were tested. Our study recruited 62 NCAA Division I college athletes. Participants provided a urine sample where researchers and participants determined the urine color of the sample based on a urine color chart and USG was measured (1). In addition, a list of all foods and beverage consumed by participants were recorded for three days. Our correlational analysis suggest that UC, even when self-assessed by the athlete, can accurately determine if NCAA division I college athletes are well-hydrated or not. In addition, our results suggest that some modification made be needed to the ACSM and NATA hydration categories.

1. Armstrong LE, Maresh CM, Castellani JW, et al. Urinary indices of hydration status. *Int J Sport Nutr.* 1994;4(3):265-279. <http://www.ncbi.nlm.nih.gov/pubmed/7987361>. Accessed October 26, 2016.

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CHAPTER 1: Introduction

Regulation of Total Body Water

The process of maintaining total body water (TBW) through water retention and thirst stimulation is regulated by the central nervous system in an intricate process. The organum vasculosum of the lamina terminalis (OVLT) is an organ located in the brain that is primarily responsible for regulating fluid status based on changes in blood plasma osmolality (1). When body water decreases and sodium concentrations in the blood increase, neurons of the OVLT sense changes in osmotic pressure and signal the hypothalamus to release arginine vasopressin (AVP). AVP stimulates the sensation of thirst and promotes water retention by the kidneys (1). As a consequence, the OVLT senses a decline in osmotic pressure and AVP secretion is inhibited. AVP plays an important role in regulating blood pressure through its actions on maintaining blood volume and direct vasoconstriction effects (1).

Another mechanism used to maintain water equilibrium and adequate blood volume is through the renin-angiotensin system. As TBW decreases, extracellular fluid volume decreases and is detected by glomerulus cells in the kidneys (2). In response, the kidneys release a proteolytic enzyme called renin. Once in the blood plasma, renin targets angiotensinogen to convert angiotensin I to the active form of angiotensin II (2). Angiotensin II helps to stimulate thirst to increase fluid intake and cause vasoconstriction (2). In addition, angiotensin II strongly stimulates aldosterone, leading to further reabsorption of sodium and fluid (2). Overall, these mechanisms work together to help maintain blood volume by stimulating thirst and subsequent fluid intake.

Body Fluid Compartments

Approximately 40-75% of the human body is comprised of water (3). The water content of different tissues varies considerably; fat-free mass is comprised of approximately 70-80% water, whereas fat tissue can be comprised of 10-40% water (4-5). Water is also a major component of the blood. Plasma comprises approximately 55% of total blood volume and plasma alone is about 92% water (6). Two thirds of total body water is located in the intracellular compartment. Extracellular water comprises the remaining third and consists of water found in plasma, within the lymph system, and interstitial spaces (7). Depending on where there is higher electrolyte concentrations, water is drawn into the intracellular and extracellular compartments by means of osmosis. Total body water is maintained within $\pm 0.2-0.5\%$ of daily body mass as long as fluid and electrolyte needs are replenished from losses (4). Total body water does not vary because of differences in age, race, or gender (4). Individuals with a greater muscle mass composition compared to fat-mass and more glycogen will experience increases in their intracellular water and increase TBW subsequently (7). Glycogen loading can increase TBW approximately 200mL because one gram of glycogen is stored with three grams of water (4). The fate of water release following glycogenolysis is unclear, but not likely to significantly alter fluid needs (4).

Effect of Dehydration and Hyperhydration on Body Fluid Compartments

For athletes, body water becomes particularly important and $\geq 2\%$ deficits leads to declines in physical and mental performance for many athletes (7). Adequate fluid intake is important to ensure water dependent functions including transporting nutrients and removing wastes, providing cellular structural integrity, regulating body temperature, maintaining blood

pressure through blood volume regulation, maintaining the body's acid-base balance, and supporting cardiovascular function (7-9). Exercise can quickly change total body water, which is why proper hydration practices are necessary for athletes (7).

Dehydration is generally defined as a loss in total body water (4,10). Hypohydration is defined as being both hyperosmotic and hypovolemic beyond normal variation (4). Dehydration most commonly occurs during exercise in the heat when water loss is not proportionate to sodium chloride losses (4). Sodium is necessary for euhydration to be reached because of its role in fluid retention and replenishment of intracellular fluids. Electrolyte losses are variable between individuals and are difficult to assess. Individuals can ensure fulfillment of electrolyte losses through eating foods, adding extra salt to foods if an athlete is known to have high sodium concentration in their sweat, or choosing sports beverages (4).

During exercise, total body water loss begins with decreases in extracellular fluid volume, primarily from plasma. As water loss continues, blood volume begins to decrease and plasma osmolality increases (1). In order to dilute the solute concentrations, intracellular water is directed to the extracellular fluid compartment. If hyperhydration occurs rapidly, extracellular fluids are replenished and urine appears clear. As a consequence, the sensation of thirst and AVP and aldosterone are suppressed. As a result, individuals no longer feel the urge to continue drinking and renal water retention is reduced, even though intracellular water and TBW is not fully replenished (1). When fluid consumption occurs more gradually, extracellular fluid compartments will equalize with intracellular fluids (11). Therefore, the urine samples will be lower in volume and a darker color than hyperhydrated urine samples even though a more complete hydration has occurred (11). Hyperhydration is particularly problematic for athletes if done before practice or competition, as they run the risk of feeling the urge to void while

exercising and display a body water deficit despite having large volumes of light-colored urine (4,11). If hyperhydration occurs during exercise, an individual may be unable to excrete this excess fluid because blood flow to the kidneys is reduced and urine production is limited (4). In addition, during exercise AVP and aldosterone secretions reduce diuresis (12).

Sources of Total Body Water Gain and Loss

Sources that contribute to total body water include approximately 600-750 mL from food, 250-350 mL from metabolic processes, and 450-2400 mL from beverages (13). Sources of fluid loss include approximately 450-1900 mL from insensible perspiration, 250-400 mL from the lungs by the process of insensible water loss, 100-200 mL from defecation, and 500-1000 mL during urination (13). In total, insensible perspiration accounts for approximately 15% of total water loss. On the skin, insensible perspiration is the process when sweat is produced on the skin's surface at an unnoticeably slow rate and once in contact with the air is evaporated. Insensible water loss occurs in the lungs when air becomes warmer and humidified in the respiratory pathway. Large volumes of dry air require more water to be humidified, thus increasing water losses (13).

At rest urination contributes to the greatest loss of body water, accounting for around 60% of water losses (13). However, during exercise, sweat accounts for the greatest loss of body water. The amount of water loss through sweat is variable because several factors influence sweat production including exercise intensity, exercise duration, and environmental conditions (13). During low-intensity exercise in a cool to moderate temperature environment, sweat could account for less than 500 mL/hr fluid loss (14). Contrastingly, an athlete exercising in a hot, humid environment could experience 90% of their daily water losses from sweat alone with

sweat losses as high as 2-3 L/hr (13,14). Sex differences are typically present regarding sweat rates and water loss with women tending to sweat less than men, thus having lower fluid losses, due to their smaller size and decreased metabolic rates during exercise tasks (4,15).

Prevalence of Dehydration in Athletes

Dehydration is common among athletes of all ages and competition levels (10,16). This high prevalence may be due to lack of hydration education, athletes underestimating the influence of hydration on performance, or an inability to consume enough fluids. College athletes are at a high risk of dehydration due to their intensity and frequency of exercise (10). In a sample of 263 NCAA Division I athletes, 2/3 of athletes were dehydrated prior to practice based on USG values ≥ 1.020 with 13% being severely dehydrated (10). Other studies involving athletes of all ages have reported similar levels of dehydration prevalence (16, 46). Men tend to be more dehydrated than women, however dehydration prevalence is consistent across time of day for practice, year of eligibility, stage of menstrual cycle, and in both developed and developing countries (10,15). Many athletes fall into a vicious cycle of dehydration where they are dehydrated going into practice, insufficiently hydrate during practice, and inadequately rehydrate after practice to achieve euhydration (17).

Dehydration can occur in hot or cold climates, but occurs more often in hot, humid environments, particularly if an athlete is not acclimated to the heat. Heat acclimatization generally takes 10-14 days (8). In the initial 3-5 days prior to acclimation, athletes experience elevated sweat rates and increased sodium losses. In response, fluid and sodium needs are temporarily higher than normal and athletes that do not consume adequate fluids will become dehydrated faster than usual (8). Athletes that are at an elevated risk for dehydration include

those participate in hot and humid environments, athletes that participate in weight-class sports and intentionally dehydrate themselves, and athletes that have multiple workouts per day (4).

Impact of Dehydration on Performance

Maintaining adequate hydration status is key to performance. Dehydration negatively effects performance by elevating heart rate, decreasing stroke volume, rapidly increasing core temperature, increasing perception of exertion, and causing undue fatigue, low blood volume, hyperosmolality, suboptimal performance, and dizziness (18, 19-21). These changes result in suboptimal performance physically and mentally. Although there is an overwhelming consensus that dehydration negatively impacts performance, some studies have noted dehydrated runners to be faster than their hydrated counterparts. Possible explanations for these contradictory findings may be due to the decreased energy cost of running with less weight being carried or not utilizing time spent consume fluids (22-24).

As body weight losses increase in the absence of adequate fluid intake, performance detriments are compounded. At a 1% loss in body weight the thirst mechanism is stimulated (7). At 2% body weight loss heart rate begins to increase, blood flow to the gut is diminished, and discomfort sets in (7). At 3%, urine production is limited and blood volume decreases (7). With 4% body weight loss, athletes are unable to maintain the physical demands at a given intensity and may experience nausea (7). At 5%, psychological deficits are apparent with decreased concentration and possible tingling in the extremities (7). At 6% body weight loss, core temperature, HR, and respiratory rate are significantly elevated. Serious symptoms occur at 7% body weight loss such as having poor balance control and migraines (7). Dizziness and labored breathing is apparent at 8% body weight loss (7). At 9% body weight loss, athletes are mentally

unaware and physical weakness is present (7). At 10% body weight loss, speech becomes slurred and muscles spasm (7). Kidney failure is present once 11% body weight loss is achieved (7).

Sweat production on the skin and vasodilation of peripheral vessels to the skin's surface are critically important for regulating the core temperature by initiating cooling through radiation, convection, and evaporation of sweat (1,4,7). Under hot, dry conditions, evaporation of sweat is the primary cooling mechanism and is responsible for up to 98% of cooling the body (4,8). Cooling by means of convection and radiation is utilized in thermoneutral environments, allowing athletes to sweat less and preserve body water (4). As dehydration ensues, blood volume decreases, resulting in decreased blood flow to the skin. Thermoregulatory strain can easily occur as decreased blood flow to the skin prohibits dissipation of heat produced, thus leading to elevated core temperatures (18,25). In an attempt to maintain cardiac output, HR increases and SV decreases (10,18). Dehydrated athletes are at a particularly high risk of thermoregulatory strain in hot, humid environments because evaporation of the sweat cannot occur and drip sweat does not provide a cooling effect (4). Hot, humid climates can limit evaporation to less than 80% (8). Dehydration effects may even negate any protection from heat acclimatization or fitness (4,8). Consuming adequate fluids at least 1-2 hours before exercise can reduce elevations in heart rate and prevent thermoregulatory strain (20,26).

With decreases in blood volume, the ability for muscles to receive adequate blood flow to provide oxygen and nutrients is limited (7). As oxygen availability in the muscles becomes limited, there is greater reliance on anaerobic energy systems. In response, lactic acid levels accumulate sooner, rate of perceived exertion increases, and onset of fatigue occurs more quickly at a given intensity (7). Cardiovascular strain may arise after as little as 2% body water loss has occurred (27). The risk for cardiovascular strain is elevated after >3% body weight loss due to

decreases in plasma volume (28). Core temperature will continue to rise if exercise continues at the same intensity and if fluids are not consumed (7). In most cases, fatigue onset and increased perceived exertion will cause an athlete to modify their intensity. Decreasing exercise intensity can help mitigate further elevations in core temperature. Exercise alone increases the body's heat production up to twenty times higher than resting levels (1). If an athlete is unable to expel this heat, core temperatures could increase as much as 1°C every 5 minutes (1). Specifically, progressive loss in 1% body weight loss increments will elevate core temperatures 0.15-0.20°C (8). With dehydration, exhaustion onsets sooner and at lower core temperatures due to decreases in heat tolerance (8).

Current hydration guidelines established by the American College of Sports Medicine (ACSM) and the National Athletic Trainers' Association (NATA) are aimed to prevent dehydration $\geq 2\%$ body weight loss (4,8). Above this threshold, the risk for heat illness development is elevated (8,25). In hot environments, athletes performing intense exercise begin to display decreased work capacity and cognitive ability prior to this 2% body weight loss guideline (4,8,29). An athlete's fitness level and heat acclimation status can help to abate dehydration's effect on performance, however reductions in performance are inevitable once 2.5% body weight loss occurs in the heat (8). Dehydration can occur quickly in hot environments and it is not uncommon for many athletes to lose 2-3% of their body weight (25). Greater reductions in body weight are required before reduction in performance occurs in thermoneutral climates. Specifically, significant decreases in muscle endurance and aerobic power have been shown with a 3-4% body weight loss (4,8,7). Hypohydration at 3-5% body weight loss is unlikely to negatively impact athletes' performance for anaerobic activity or activity primarily relying on muscular strength (4).

Water Recommendations

The Institute of Medicine (IOM) recommends a daily water intake of 3.7 L for men and 2.7 L for women over the age of 19 (5). Athletes fluid requirements vary depending on environmental conditions and the nature of their training. As such, the general recommendations set by the IOM should serve as a baseline and be modified to meet individual needs (5). Fluid needs may be estimated based on 1 ml per kcal consumed. This estimation is unlikely to support fluid needs in cases of intense training or exercise in the heat and humidity (30). The IOM indicates that individuals can stay sufficiently hydrated by relying on thirst as a driver for daily fluid intake and by consuming beverages at meals (5). However, athletes must consume fluids prior to and more often than when experiencing thirst to ensure euhydration (8,31).

Contributors to Daily Fluid Consumption

Consuming sufficient water daily can be achieved through fluid and food consumption. A small percentage of “metabolic water” contributes to total body water and is produced when macronutrients are broken down during aerobic metabolism (7). Consumption of fluids accounts for approximately 70-80% of water intake and foods for the remaining 20-30% (5,7). Solid foods water content varies widely with meats, dairy, and grains containing 30-50% water and vegetables and fruit containing 70-90% (7). Foods are also important for fluid retention and electrolyte replenishment (4). Current evidence suggests that macronutrient composition has little influence on urinary losses and is unlikely to alter fluid requirements (4). There is some evidence that long term (24 months) low-carbohydrate, high-protein diets in combination with weight loss leads to greater urinary losses through increased excretion of ketones and urea (32). More evidence is needed to confirm this link before recommending increasing fluid intake for

athletes on a high protein diets. There is no evidence linking short-term, high-protein diets and elevated urinary excretion volume (32).

Before Exercise

Prior to exercise, fluid intake should occur several hours before exercise in the form of both fluids and meals to ensure proper fluid absorption and normal urine output levels (4). Specifically, at least 4 hours prior to activity, athletes are recommended to consume 5-7 mL/kg body weight or 400-600 mL of fluids (4,8). If the athlete is experiencing a lack of urine production or has dark urine 2 hours before exercise, they should continue to slowly consume additional fluid. 10-20 minutes before exercise athletes should consume 200-300 mL of fluids (4,8). In order to ensure fluid retention and thirst simulation, athletes are recommended to eat sodium containing foods or choosing beverages with 20-50 mEq of sodium (4,33).

During Exercise

The goal of hydration during exercise is to prevent $\geq 2\%$ body weight loss, maintain plasma volume, and prevent large electrolyte losses (4,7-8). Individual hydration plans should be established in order to accommodate individual sweating rates, exercise intensity and duration, heat acclimatization state, metabolic efficiency, environmental conditions, and sweat electrolyte concentration (4,33). Sweat rates are highly variable among athletes, even within the same sport and climate, which is why a one-size-fits-all hydration plan is not appropriate (4). Ideally, athletes should consume enough fluids to match sweat and urine output volumes. Consuming 7-10 fl oz every 10-20 minutes is enough for most athletes to achieve hydration goals during

exercise (4,8). Most athletes are able to tolerate up to 1 L of fluids per hour based on gastric emptying rates, but most consume <500 mL/hr (34).

Some athletes may choose caffeinated beverages to preserve exercise performance (4). Current evidence indicates that small doses of caffeine are unlikely to significantly increase urine output and caffeinated beverages can contribute to total daily fluid intake (4-5). As long as athletes are drinking additional non-caffeinated fluids and are eating meals normally, consuming caffeine, even in moderate amounts, is unlikely to drastically alter fluid balance (25).

After Exercise

Athletes should determine body water losses through pre and post weighing and should consume fluids to match 100-150% of these losses. Specifically, one pound of body weight lost equates to 16-24 fl oz or for each kg lost 1-1.5 L of fluid is needed to adequately rehydrate (4). In times where rapid recovery is necessary, an athlete should begin their recovery fluid consumption as soon possible (35). Sodium consumption during the recovery period in the form of food or hypertonic beverages is important to ensure fluid retention and to further stimulate thirst (1,4). If an athlete does not consume sodium while replacing fluid losses, they will be unable to achieve a euhydrated state (4). ASCM does not recommend alcohol for rehydration post-exercise due to its strong diuretic effect (4).

Methods of Hydration Assessment

Hydration can be assessed using several techniques such as urine osmolality, plasma osmolality, urinary specific gravity (USG), body weight changes, urine color, and urine volume. Measurements of plasma osmolality with isotope dilution methods of total body water are the

gold standard for assessing an individual's hydration status, however it is not feasible assessing athletes daily (4,15). Of the urinary biomarkers, USG and urine osmolality are objective measurements that are most valid in determining hydration status (4). Plasma osmolality is less likely to detect mild dehydration compared to the urinary biomarkers (28). Total body water, plasma osmolality, and body weight are biomarkers that can indicate both acute and chronic changes in hydration (4). USG and urine osmolality are valid for assessing only chronic changes, as these measures are less accurate in acute timeframes, especially when volumes of hypotonic fluids are consumed rapidly (4). Urine color and urine volume have also been deemed valid biomarkers for determining hydration status in a variety of settings, but are not recommended when high precision is required (28).

The ASCM deems hydration biomarkers appropriate for athletes if they are able to detect $\geq 3\%$ change in total body water and are practical for coaches and athletes to use (4). Practicality includes being cost-effective, time-efficient, and does not require technical expertise for use (4). The most practical hydration indices athletes can use are USG, urine osmolality, body weight, urine volume, thirst, and urine color (4,33). Thirst should not be solely relied upon to indicate adequate hydration, being it occurs once an athlete is already hypohydrated by 1% body weight loss. If possible, multiple practical biomarkers should be used together to increase the validity of the hydration measurement rather than relying on a single biomarker alone (4, 36-37).

USG

USG is a hydration biomarker taken using a refractometer and measures the mass and number of solutes present in urine (11). A refractometer is a relatively simple tool to use, but they can be expensive and most athletes' have limited to no access to one on a daily basis (8).

For non-athletes, some research has defined hyperhydrated values by USG values 1.001-1.012; euhydration 1.013-1.029; and dehydration ≥ 1.030 (38). Hydration guidelines established by ASCM for athletes define dehydration >1.020 (4). Research has demonstrated a body mass loss of 2% with USG values >1.020 - ≥ 1.025 (32,39). USG is one biomarker that can be confounded if hyperhydration occurs in a short period of time, resulting in a false display of euhydration values (1,4,11). Testing first morning void or several hours once stable hydration status has been reached can ensure accurate USG values (4).

Urine Osmolality

Urine osmolality is a hydration biomarker that measures the number of solutes present in the urine (11). In an athletic setting, urine osmolality is the least practical urinary biomarker because it requires using freezing point depression (40). In non-athlete populations, urine osmolality values <800 mOsm indicate euhydration (40). In athletes, urine osmolality values ≤ 700 mOsm indicate euhydration; values 700-900 mOsm indicate mild to moderate dehydration; and values ≥ 900 mOsm indicate moderate to severe dehydration (4,17,31). Often in research, USG and urine osmolality are used interchangeably based on their high correlation ($r=0.97$) (11,28). However, if USG values are >1.024 or urine osmolality values >900 mOsm/kg, the correlational strength weakens (11,28,31). In a lab setting if the most accurate hydration assessment is required using urinary biomarkers, urine osmolality should be used over USG because it is not affected by solutes present in the urine, such as glucose, protein, or urea (28,31).

Body Weight

Body weight change is a hydration biomarker that should be used with urinary biomarkers to provide a more valid indication of hydration status (4). This measurement can be used either by measuring daily morning weight or by weighing pre and post exercise to determine body weight loss. If taking daily morning weight, an athlete should get a baseline body weight by taking their weight in the morning for three consecutive days after first void (4). To achieve the highest validity of this measurement, athletes should weigh themselves at a consistent time and after their first void to prevent body weight changes based on food intake, bowel movement patterns, and differences in bladder fullness (4,7). Athletes in energy balance should only fluctuate body weight approximately 1% (4). However, during the luteal phases of the menstrual cycle women commonly experience an increase in total body water (4). To ensure accuracy, baseline body weight measurement need to be determined frequently if an individual is experiencing body composition changes (4).

Athletes weighing themselves pre and post exercise are able to measure body weight loss through sweat, and in response can determine how much fluid is required to sufficiently rehydrate post-exercise. With this approach it is assumed that 1 gram of body weight lost is due to 1 mL of sweat lost (4). For most exercise events, decreases in body mass are unlikely to be due to body tissue catabolism. This method should not be used for athletes participating in ultra-endurance events because body weight loss cannot be assumed from water loss alone (12).

Hydration Status Cut-Points

NATA distinguishes hydration status of athletes into several categories including well-hydrated, minimal dehydration, significant dehydration, and serious dehydration based on

criteria for body weight change, urine color, and USG (8). Well-hydrated athletes have $\leq 1\%$ body weight fluctuations, urine color of 1-2 based on an 8 scale color chart, or have USG values < 1.010 ; minimal dehydration is categorized by 1-3% body weight loss, urine color of 3-4, or USG values 1.010-1.020 (8). With significant dehydration, body weight losses are at 3-5%, urine color at 5-6, or USG at 1.021-1.030.⁸ Body weight loss $> 5\%$, urine color > 6 , or USG > 1.030 indicate serious dehydration (8). ACSM hydration guidelines are more general than NATA and categorize euhydration with plasma osmolality < 290 mOsmol, USG ≤ 1.020 , urine osmolality < 700 mOsmol, and body weight loss $< 1\%$.¹ Athletes should aim to stay at or below the minimal dehydration (8).

Urine Color

Urine color is a hydration biomarker that is primarily due to urochrome concentrations in the urine. Urochrome is a byproduct of hemoglobin breakdown that produces the yellow color in urine and is influenced by both solute and water presence (11,40). Urine color tracks changes in body water similar to urine osmolality, USG, and urine volume (11). To determine hydration status, athletes compare their urine to a urine color chart. In the absence of a urine color chart, athletes should aim to have urine color that is “very pale/pale yellow” or “straw colored” to ensure euhydration (28). Dark colored urine indicates that an athlete is dehydrated, whereas light colored urine indicates a euhydrated state (4,41). Assessing hydration status using urine color is inexpensive because all that is required is access to a urine color chart. If urine collection cups are used the cost becomes expensive over time (8).

Urine color is one biomarker that can be confounded if hyperhydration occurs in a short period of time, resulting in a false display of euhydration from light colored urine (11). True

hydration status can be determined several hours after hyperhydration occurs once rehydration is slowed (41). Similarly, urine color may be influenced by the presence of blood and some supplements and prescription drugs that change urine color (11,31,41,43).

Urine Color Analysis.

This most widely used urine color chart is an eight-color scale with colors that range from a pale, very lightly colored yellow to brown-green (28). The specific colors used in the color scale were chosen from standardized colors from A. Maerz and M. Rea Paul *Dictionary of Color* (2nd edition), a book about compendium of colors (28,44). For urine color analysis, urine is collected in a urine collection cup and this sample is held next to the urine color scale and against a white background in a well-lit room (28). Urine color of the sample is chosen at the whole number that most closely resembles a color on the urine color chart (40,45). It is best technique to get a clean catch, mid flow urine sample in order to provide consistency in the technique and improve accuracy of the measurement (8, 15,31). Ideally, urine specimens should be analyzed ≤ 30 minutes of collection (28). In research settings, the same investigator should determine urine color of all samples or inter-rater reliability needs to be established.

Urine Color Validity

Urine color has been reported to moderately- strongly correlate with USG and urine osmolality, but is not significantly correlated with plasma osmolality, plasma sodium, or hematocrit (28,31,40). Current research has shown urine color to correlate moderately with urine osmolality using a Spearman's rank-order correlation in athletes under euhydration conditions with a correlational coefficient ($r=0.743$), however the strength of this correlation decreases as

dehydration increases (31). Specifically, in athletes who are severely dehydrated, the strength of this correlation is low ($r=0.498-0.398$) (31). In distinguishing between euhydration and dehydration, a urine color of ≥ 5 after exercise in the heat can identify body mass loss $\geq 2\%$ with 88.9% sensitivity and 84.8% specificity (45).

There is some debate regarding which urine void provides the most valid indication of hydration status based on urine color. Current ASCM guidelines recommend using the first morning void or samples after stable hydration has occurred over numerous hours (4). A major advantage to using the first morning void is that there has been no food or fluid intake and no sweat loss has occurred (10). Others believe the second void urine color more accurately predicts of hydration status because first daily void tends to be darker in color and is more concentrated than later voids (28). To our knowledge no research has tested which void has superior validity.

Validity of Urine Color Self-Assessment

Athletes' ability to accurately self-assess urine color enables this tool to be useful in the absence of healthcare professionals. Two studies have assessed youth athletes ability to self-assess urine color, but to our knowledge no studies have determined the validity of urine color self-assessment in college athletes (40,43). In youth athletes, a majority are able to determine if their urine indicates dehydration but are unlikely to correctly identify specific urine color numbers (40). Correctly self-assessing euhydrated is more challenging (40). When investigating USG and researcher determined urine color, Pearson's product-moment correlation coefficients have been reported at ($r=0.82$) (43). USG and subject self-assessed urine color have shown lower correlations at ($r=0.60$) (43). Most accurate dehydration self-assessment in youth occurred if the urine was ≥ 4 color on the 8 color urine chart (40). To ensure the highest validity for urine color

self-assessment of all ages, it is recommended to train athletes on how to use the urine color chart properly and interpret the values (43).

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CHAPTER 2: Validation for Measures of Hydration Status in College Athletes

ABSTRACT

Purpose: The commonly used urine color chart has been validated in several populations, but not NCAA division I athletes from a variety of sports. In addition, the American College of Sports Medicine (ACSM) and the National Athletic Trainers' Association (NATA) provide indexes of hydration status/ euhydration cut-off for various hydration indices. However, to our knowledge no prior studies have assessed the utility of these hydration indexes in NCAA division I college athletes. The primary purpose of this study is to validate urine color as a measurement of hydration status and evaluate the utility of urine color and USG with NATA and ACSM indexes of hydration status/euhydration cut-off in NCAA division I college athletes. **Methods:** Sixty-two NCAA division I college athletes (25 males, 37 females) volunteered to participate. Participants provided a urine sample ≤ 30 minutes prior to exercise for urine color self-assessment and researcher assessment and urine specific gravity (USG) analysis. In addition, three 24-hour recalls were obtained on nonconsecutive days. **Results:** Self-assessed and researcher-assessed urine color displayed a moderately strong positive relationship to USG ($r=0.679$, $p<0.0001$) and ($r=0.772$, $p<0.0001$), respectively. Participants self-assessed urine color similarly to researchers ($r=0.859$, $p<0.0001$) with strong agreement. Urine color and USG are independent of hydration index/euhydration cut-off established by ACSM and NATA. **Conclusion:** Urine color by use of the urine color chart is a valid method for assessing hydration status in NCAA division I college athletes from a variety of sports. Urine color assessment does not always identify the appropriate hydration index/euhydration cut-off established by ACSM and NATA for USG. **Key Words:** NCAA division I college athletes, hydration status, urine color, urinary specific gravity, dehydration.

INTRODUCTION

Fluid requirements of collegiate athletes are unique due to training and competition, travel, school schedules, and stressors common in college environments. Adequate hydration is critical for optimal performance and health in NCAA Division I college athletes. Unfortunately, dehydration is common among athletes of all ages. More than half of NCAA division I collegiate athletes have been reported hypohydrated prior to practice (1). Athletes may fall into a cycle of dehydration where they arrive to practice dehydrated, inadequately hydrate during exercise, and insufficiently rehydrate fluid losses the remainder of the day (2). Current hydration guidelines established by the American College of Sports Medicine (ACSM) and the National Athletic Trainers' Association (NATA) are aimed to prevent dehydration $\geq 2\%$ body weight loss due to performance and health reductions beyond this threshold (3-4). However, athletes performing intense exercise in the heat may exhibit reduced work capacity prior to 2% body mass loss (3-5).

In athletic environments, urinary biomarkers are useful for identifying an athlete's hydration status. Having practical and valid methods to assess hydration status allows athletes to identify hypohydration and subsequently adjust fluid intake to ideally achieve euhydration. Serum osmolality is the gold standard for hydration assessment but is impractical for use in athletic settings (3,6). Common urinary hydration biomarkers used in field settings include body weight changes, urine osmolality, urine color, and urinary specific gravity (USG). Current hydration guidelines define euhydration by $\leq 1\%$ body weight fluctuations, urine osmolality < 700 mOsmol, urine color of 1-2 based on an 8 scale color chart, and USG values < 1.010 (4). Several studies have used urine color ≤ 3 as the cut off for adequate hydration (7-10).

USG measures the mass and number of solutes present in urine using a refractometer (11). Hyperhydration in a short period of time or solute presence, such as glucose, protein, or

urea can confound this measure (3-4,7,11-13). In many athletic settings, athletes have access to a urine color chart. The eight-color urine color chart ranges in colors from a pale, very lightly colored yellow to brown-green (7). Urine color is confounded by hyperhydration in a short period of time, the presence of blood, and by some medications and supplements (11,13-15).

To assess urine color, urine samples are compared to the validated, urine color chart (7). To our knowledge, use of this urine color chart has not been validated in NCAA division I collegiate athletes, with limited validation only occurring with college males and NCAA Division I tennis athletes (7). In addition, to our knowledge no studies have validated urine color self-assessment in this population. With hydration being a critical component to athletes' success and health, it is important to validate practical tools for hydration assessment in this population. The aims of this study are to 1) validate urine color as a measurement of hydration status in NCAA Division I college athletes, and 2) evaluate the utility of urine color and USG with NATA and ACSM indexes of hydration status/euhydration cut-off in NCAA division I college athletes. Our hypotheses are that urine color will accurately reflect the hydration status of NCAA Division I collegiate athletes and that the assessment of urine color will classify the hydration status based on NATA and ACSM indexes of hydration status/euhydration cut-off similar to USG in NCAA Division I college athletes.

MATERIALS AND METHODS

Participants

A sample of 62 NCAA division I collegiate athletes (25 men, 37 women; age range, 18-23 years) from men and women's tennis, swimming, volleyball, hi-tech dance team, men's basketball, men and women's cross-country, men and women's track and field, men and

women's soccer, and football volunteered to participate in this observational investigation. Eligibility criteria included being 18 years or older and a member of an NCAA division I collegiate team at Virginia Tech or Radford University. Data collection occurred April-October, 2017, with a majority of participants in their offseason. Participants with injury or acute illness that prevented them from participating in regular team training and/or competition and individuals with metabolic conditions that affected normal urinary processes were excluded from the study. The investigation was approved by the Institutional Review Board at Virginia.

Recruitment

Participants were recruited via email and flyers distributed to NCAA sports teams at Virginia Tech and Radford University. Brief meetings were organized with individual teams to detail the study's purpose and procedures. At this meeting participants were provided with a written informed consent form to review. Signed informed consent forms were gathered after the recruitment meeting or at the first visit.

Experimental Procedures

This investigation was delivered over a one-two week period where participants completed three visits on nonconsecutive days. The schedule of study visits is illustrated in Figure 1. During the first visit, participants completed a brief, general information and health questionnaire to provide information regarding gender, age, year of eligibility, sport, and role on the team. Height, body weight, and computed BMI measurements were gathered. Body weight was measured using a digital scale accurate to +/-0.1 kg (Scale-Tanita Bf-350) with participants

wearing light clothing and no shoes. Height information was taken from official athletic rosters online based on yearly physicals.

In addition, midstream urine was collected into a clean urine collection cup ≤ 30 minutes prior to exercise. If an athlete was unable to provide a urine sample at the first visit, the sample was collected at one of the subsequent visits. Participants were instructed to self-assess their urine sample by comparing this sample with the eight-color urine color chart against a white piece of paper in a well-lit room (7). Colors are numbered in ascending order with shades that range from a pale, very lightly colored yellow to brown-green (7). Colors 1-2 were interpreted as well-hydrated, colors 3-6 as mild to moderately dehydrated, and colors ≥ 7 as severely dehydrated. Participant urine color self-assessments (UC_{sub}) were concealed from researchers until the completion of urinary analysis.

At all three visits a 24-hour recall was performed using the USDA Automated Multiple-Pass Method (16). In total, two weekday recalls and one weekend day recall were taken. These recalls were performed at a location at the athletic campus (i.e. practice facility, weight room, etc.). Participants listed all food and beverages consumed the prior day. Food diagrams were used to assist participants in recalling the appropriate portion sizes. The dietary data was analyzed using NDS-R version 2011 (University of Minnesota, Minneapolis MN).

Investigator Urine Analyses

Participant urine samples were analyzed by researchers for urine color and USG ≤ 30 minutes of collection time. Researcher determined urine color (UC_{res}) was assessed in the same location and manner as instructed to participants. Multiple researchers collaboratively determined the urine color of the sample when possible, or one researcher would do so in the absence of other

investigators. USG was measured in duplicates using a calibrated digital refractometer (± 0.001 ; Atago, 4410 PAL-10S, Tokyo, Japan) and 2-3 teaspoons of the urine sample.

STATISTICAL ANALYSIS

Spearman's correlation analysis was executed to examine the relationship between USG and UC_{res} , and UC_{sub} and UC_{res} . The mean of duplicate USG measures was used in statistical analysis. In addition, a Bland-Altman plot was performed to evaluate the agreement between UC_{sub} and UC_{res} . Chi-square (X^2) analysis was used to assess the association between our hydration indices and the indexes of hydration status/euhydration cut-off for USG and urine color established by ACSM and NATA (3-4). ACSM urine color euhydration cut-off was based on NATA urine color categories for USG of ≤ 1.020 to indicate euhydration (urine colors 1-4) and ≥ 1.021 to indicate dehydration (urine colors 5-8) (4). Statistical significance was set at $p < 0.0001$. All statistical analysis was performed using IBM SPSS Statistics 24.

RESULTS

Participant characteristics of the 62 subjects are presented in Table 1 and habitual dietary intake including total energy, macronutrient, alcohol, caffeine, and water intake are located in Table 2. The mean values and ranges of USG and urine color in males, females, and the pooled sample are presented in Table 5. Table 5 also details the average USG associated with each urine color, based on UC_{res} values. The frequency of participants in each of the NATA and ACSM indexes of hydration status/euhydration cut-off based on urine color and USG are represented in Tables 6-7.

Validity of urine color as an indicator of hydration status

Spearman rho correlation analysis revealed that UC_{res} and UC_{sub} were significantly correlated to USG, ($r=0.772$, $p<0.0001$) and ($r=0.679$, $p<0.0001$), respectively. Overall, urine color displayed a moderately strong positive relationship as a predictor of USG in NCAA division I collegiate athletes.

Self-assessment of urine color

Spearman rho correlation analysis revealed that UC_{res} and UC_{sub} were significantly correlated to one another ($r=0.859$, $p<0.0001$). A Bland-Altman plot was used to assess the agreement between UC_{res} and UC_{sub} in Figure 2. The frequency of the difference ($UC_{sub}-UC_{res}$) for each coordinate's repetition is illustrated in a corresponding bar chart for the Bland-Altman plot in Figure 3. Overall, urine color rating for researchers and subjects displayed a strong positive relationship with strong agreement.

Utility of urine color and USG using the NATA and ACSM hydration indexes

The mean values and ranges of USG values for UC_{res} range in Table 5 illustrate discrepancies between our participants and the hydration indexes/euhydration cut-off of NATA and ACSM. Specifically, the average USG for urine colors 1-2 in our data fell outside of the NATA recommendations for an athlete being well-hydrated. All other average USG values for urine colors fell into the appropriate hydration indexes established by NATA and ACSM. USG value ranges for all urine colors would not classified participants into the appropriate NATA and ACSM hydration indexes.

Chi-square analysis of our hydration indices and the indexes of hydration status/euhydration cut-off from NATA and ACSM were not significant (Tables 6-7). Specifically, hydration biomarkers and NATA indexes of hydration status displayed $X^2= 1.3726$, $p=0.851$; hydration biomarkers and ACSM euhydration cut-off displayed $X^2= 0.774$, $p=0.683$. The non-significance of the chi-square tests indicate that 1) urine color and USG are not significantly associated with the hydration status categories from ACSM and NATA in NCAA division I collegiate athletes, and 2) there is no significant difference in the frequency of urine color and USG among all hydration indexes.

Gender Differences in Hydration Status

As illustrated in Table 5, a majority of athletes (50.0%) displayed euhydration with USG values 1.010-1.020 and urine colors 3-4. 27.4% of participants displayed USG values ≥ 1.021 (14.5% males, 12.9% females) and 22.6% of athletes displayed urine colors ≥ 5 (11.3% males and females) based on UC_{res} . No athletes displayed USG values ≥ 1.030 or urine colors ≥ 7 . Gender differences were minimal among dehydrated samples, but a greater percent of females were most hydrated. Specifically, 16.1% of females compared to 6.5% of males displayed USG values < 1.010 , and 16.1% of females compared to 11.3% of males displayed urine colors 1-2.

DISCUSSION

The main findings of this investigation was that there was a strong positive correlation between USG and urine color. In addition, the USG based on urine color is not consistent with hydration/dehydration categories established by ACSM and NATA (3-4). Distinguishing between well-hydrated and minimal dehydrated categories lends itself to be the most difficult

and least accurate from the hydration indices tested. Taken together, our findings support the application of the urine color chart, including for athlete self-assessment, in NCAA division I college athletes from a variety of sports. However, some modification of the ACSM and NATA hydration categories may be warranted. To our knowledge, no prior studies have investigated urine color self-assessment in college aged individuals or the utility of NATA indexes of hydration status and ACSM euhydration cut-points based on urine color and USG (3-4).

This study expanded on the previous work of Lawrence Armstrong, et al. (1994), Fernandez-Elias et al. (2014), and Kavouras (2016) examining the relationship between USG and urine color (7,9,13). Our displayed results for urine color illustrating a moderately strong positive relationship to USG supports our first hypothesis that urine color accurately reflects the hydration status of NCAA Division I collegiate athletes. This finding is consistent with other research. Our lack of significance from the chi-square analysis determines that the assessment of urine color did not classify the hydration status based on NATA and ACSM indexes of hydration status/euhydration cut-off similar to USG in NCAA Division I college athletes

Armstrong, et al. (1994) assessed the utility of hydration biomarkers in college ages males and NCAA division I tennis athletes (7). There was a moderate positive correlation between urine color and USG in college males ($r=0.54$, $p<0.01$); a strong positive correlation between urine color and USG in college males after exercising in the heat ($r=0.92$, $p<0.0001$); and a moderate positive correlation between urine color and USG in NCAA division I tennis athletes prior to exercise ($r=0.78$, $p<0.0005$) (7). A strong positive correlation between urine color and USG was reported from the combined subject analysis ($r=0.80$, $p<0.0001$) (7). Our investigation reports nearly identical correlations between urine color and USG to the NCAA division I tennis athletes of Armstrong, et al. (1994) (2). Similar correlations to our investigation

have been reported between urine color and urine osmolality under euhydrated samples of Olympic combat athletes and healthy 8-14 year old children, ($r=0.743$, $p=0.000$) & ($r=0.45$, $p<0.001$), respectively (9,13).

Healthy children and adolescent youth have been reported to accurately self-assess their urine color similar to researchers 67-78% of the time with a moderate correlation between self-assessed urine color and USG ($r=0.60$, $p<0.001$) (9,14). Our findings of the strength of the relationship between researcher determined urine color and USG being stronger than the relationship between subject self-assessed urine color and USG is consistent with the youth studies (9,14). Children do not reliably self-assess euhydrated samples and most similarly self-assess their urine as researchers when urine color is ≥ 4 (9). The similarity between participant and researcher correlation to USG across all levels of hydration in our investigation is likely due college athletes being educated how to properly use the urine color chart and through years of experience of self-assessing their urine with a urine color chart. In addition, the importance of staying well hydrated reiterated to college athletes more often than to their youth counterparts.

ACSM euhydration cut-points are likely to be most useful for NCAA division I college athletes. The NATA urine color recommendations for urine colors 1-4 having a USG of ≤ 1.020 could be a tool some athletes may use in times when refractometers are not readily available. The addition of other biomarkers of hydration, such as body weight changes, may be helpful for athletes in assessing their hydration status (3).

In a prior study, 66% of NCAA division I college athletes were hypohydrated prior to practice based on USG values ≥ 1.020 ; and additionally 13% were severely dehydrated (USG values ≥ 1.030) (1). A greater percentage of the hypohydrated athletes were reported to be males (1). Even at the most elite levels of athletes, dehydration is common. Osterberg et al. (2009)

demonstrated that among 29 NBA players from 5 teams, 52% of athletes were dehydrated within an hour before a game with USG values >1.020 (17). The lower prevalence of dehydration in our study may be attributed to having the greater number of female participants (1). The latter observation is consistent with prior studies (1,6).

Strengths

There are several strengths of our study. First, athletes from a variety of sports participated in our investigation. Second, there was good representation of both genders (25 males, 37 females). Taken together, our findings would appear to be generalizable to many NCAA Division I college athletes. Second, we utilized a valid and objective measurement of USG, i.e. digital refractometer. Finally, participants and researchers urine color assessment were independently determined and USG analysis occurred subsequently so as to not influence the former.

Limitations

There are some limitation of our study which should be acknowledged. First, we did not control for potential factors that may have influenced urine color and USG, such as prior training, fluid intake, supplement and medication use. ACSM recommends most accurate distinguishing of euhydration and dehydration to occur using the first morning void or once stable hydration status has been reached for several hours (3). Second, we did not screen participants for color blindness. None were reported and some may have been unaware of being color blind (18). Third, interrater reliability was not established between researchers assessing urine color of urine samples. Establishing interrater reliability would ensure consistency in urine color assessment technique and ratings. Fourth, we did not have participants that were classified

into highest categories of dehydration, specifically no subjects had a urine color 7-8 or USG value >1.030 . A wider range of hydration status is needed to provide valuable insight to urine color validity at severely dehydrated states. Fifth, our sample included athletes of a healthy and overweight status based on BMI. The study lacks generalizability to NCAA Division I college athletes from our included sports whose BMI classifies them as underweight or obese. Finally, urine color and USG do not provide any quantification for how much fluid intake would be needed to rehydrate in individuals that are dehydrated. As such, their utility must be considered with this in mind.

FIGURES

Figure 1. Schedule of study visits.

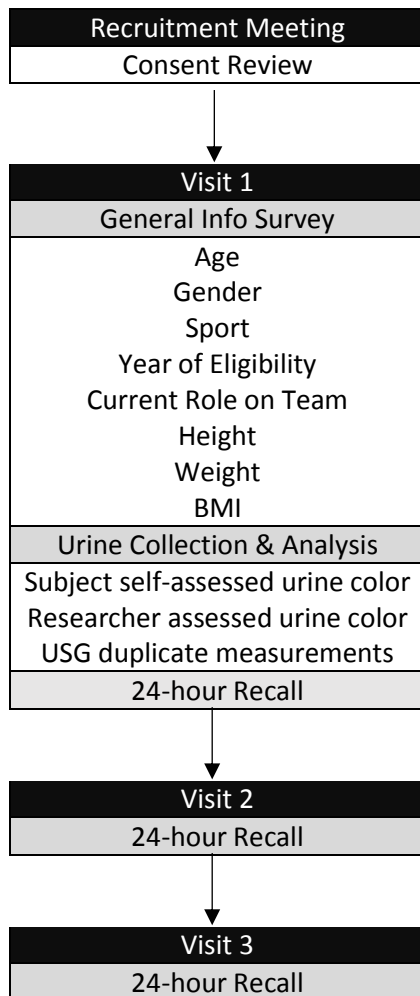


Figure 2. Bland-Altman plot comparing researcher assessed urine color from subject self-assessed urine color.

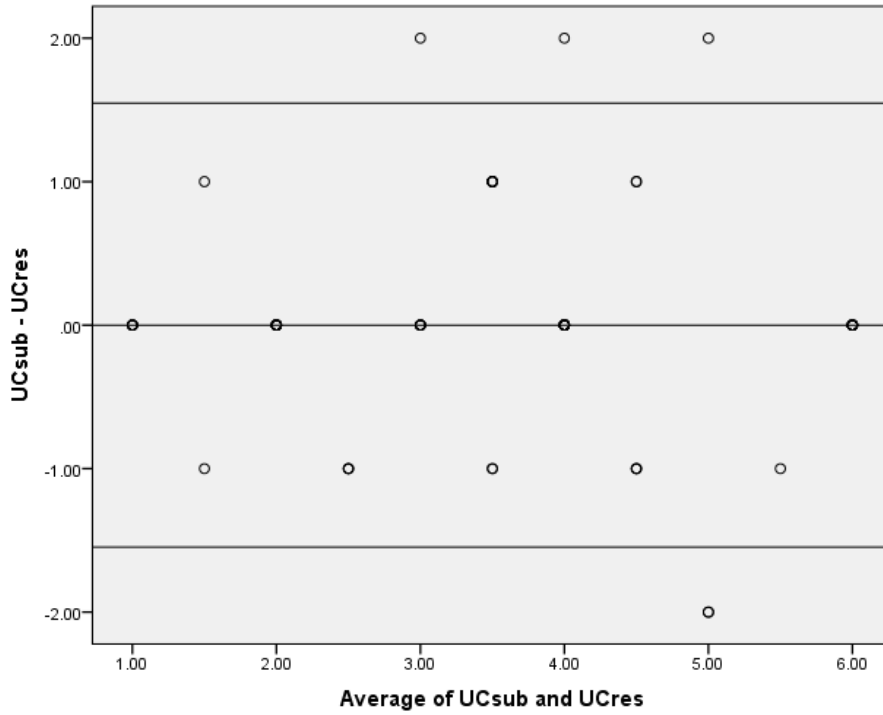
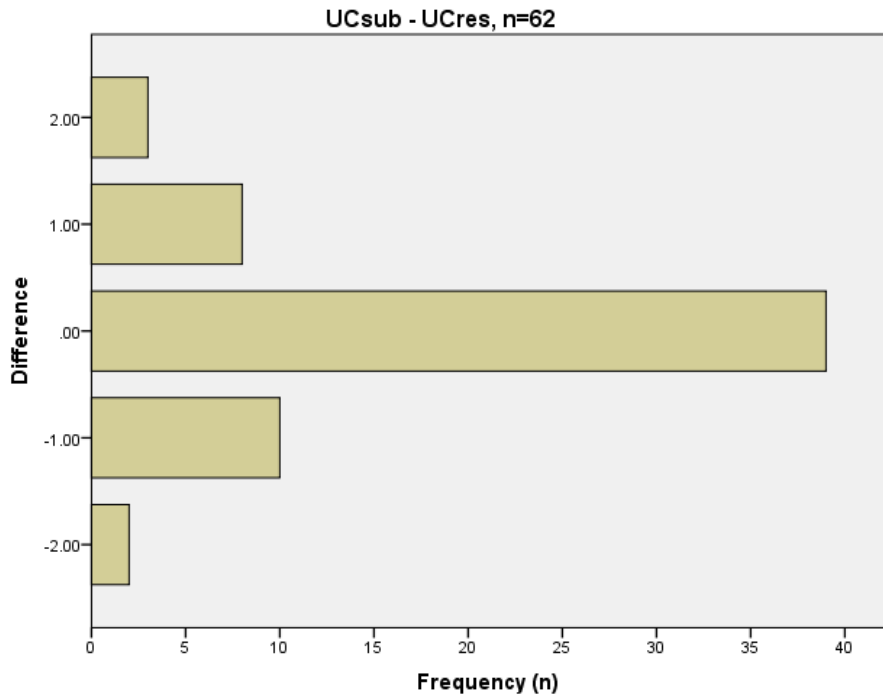


Figure 3. Frequency of the difference in researcher assessed urine color and subject self-assessed urine color. Multiple data points are represented by each dot on the chart.



TABLES

Table 1. Participant Characteristics

Participant Characteristics	Total	Male	Female
Total # of participants, n (%)	62	25 (40.3%)	37 (59.7%)
Age (y)	19.8 ± 1.5	20.4 ± 1.6	19.4 ± 1.4
18, n (%)	16 (25.8%)	3 (4.8%)	13 (21.0%)
19, n (%)	14 (22.6%)	6 (9.7%)	8 (12.9%)
20, n (%)	10 (16.1%)	3 (4.8%)	7 (11.3%)
21, n (%)	13 (21.0%)	8 (12.9%)	5 (8.1%)
22, n (%)	6 (9.7%)	2 (3.2%)	4 (6.5%)
23, n (%)	3 (4.8%)	3 (4.8%)	0 (0%)
BMI (kg/m ²)	22.7 ± 1.9	23.3 ± 2.3	22.3 ± 1.5
BMI categories:			
Obese, n (%)	0 (0%)	0 (0%)	0 (0%)
Overweight, n (%)	6 (9.7%)	5 (8.1%)	1 (1.6%)
Healthy Weight, n (%)	56 (90.3%)	20 (32.3%)	36 (58.0%)
Underweight, n (%)	0 (0%)	0 (0%)	0 (0%)
Year of eligibility	2.5 ± 1.3	2.6 ± 1.2	2.5 ± 1.3
1=Freshmen, n (%)	18 (29.0%)	6 (9.7%)	12 (19.3%)
2=Sophomore, n (%)	14 (22.6%)	6 (9.7%)	8 (12.9%)
3=Junior, n (%)	14 (22.6%)	7 (11.3%)	7 (11.3%)
4=Senior, n (%)	13 (21.0%)	5 (8.1%)	8 (12.9%)
5=Red shirt senior, n (%)	3 (4.8%)	1 (1.6%)	2 (3.2%)
Sport			
Tennis	10 (16.2%)	6 (9.7%)	4 (6.5%)
Cross country	8 (13.0%)	4 (6.5%)	4 (6.5%)
Swimming	3 (4.8%)	0 (0%)	3 (4.8%)
Baseball	2 (3.2%)	2 (3.2%)	0 (0%)
Volleyball	8 (12.9%)	0 (0%)	8 (12.9%)
Basketball	3 (4.8%)	3 (4.8%)	0 (0%)
Dance	1 (1.6%)	0 (0%)	1 (1.6%)
Soccer	23 (37.1%)	8 (12.9%)	15 (24.2%)
Track and field	3 (4.8%)	1 (1.6%)	2 (3.2%)
Football	1 (1.6%)	1 (1.6%)	0 (0%)

Values are mean ± SD (unless otherwise indicated)

Table 2. Habitual Dietary Intake

Calories (kcal/day)	2676 ± 784.2 (1248-4765)
Fat (g)	102 ± 37.3 (39-193)
Carbohydrate (g)	329 ± 95.5 (132-564)
Protein (g)	115 ± 39.7 (56-232)
Alcohol (g)	4 ± 10.9 (0-55)
Calories from fat (%)	33 ± 4.8 (20-49)
Calories from carbohydrate (%)	49 ± 6.5 (30-63)
Calories from protein (%)	17 ± 3.4 (11-30)
Caffeine (mg)	58 ± 67.9 (0-310)
^a Water (g)	4250 ± 1317.9 (1922-8466)

^aWater grams is from fluid intake and water in food.

Values are mean ± SD (range), unless otherwise indicated

Table 3. American College of Sports Medicine Euhydration Cut-off for USG (3)

Hydration Status	USG
Euhydration	≤1.020
Dehydration	≥1.021

Table 4. National Athletic Trainers' Association Indexes of Hydration Status for Urine Color and USG (4)

Hydration Status	Urine Color	USG
Well-hydrated	1-2	<1.010
Minimal dehydration	3-4	1.010-1.020
Significant dehydration	5-6	1.021-1.030
Severe dehydration	7-8	>1.030

Table 5. Urine Color and USG in Males, Females, and the Pooled Sample

Urinary Characteristics	Total	Male	Female
UC _{sub}	3.5 ± 1.5 (1-6)	3.6 ± 1.5	3.5 ± 1.6
1-2, n (%)	19 (30.6%)	7 (11.3%)	12 (19.3%)
3-4, n (%)	30 (48.4%)	12 (19.4%)	18 (29.0%)
5-6, n (%)	13 (21.0%)	6 (9.7%)	7 (11.3%)
7-8, n (%)	0 (0%)	0 (0%)	0 (0%)
UC _{res}	3.5 ± 1.5 (1-6)	3.8 ± 1.5	3.4 ± 1.5
1-2, n (%)	17 (27.4%)	7 (11.3%)	10 (16.1%)
3-4, n (%)	31 (50.0%)	11 (17.7%)	20 (32.3%)
5-6, n (%)	14 (22.6%)	7 (11.3%)	7 (11.3%)
7-8, n (%)	0 (0%)	0 (0%)	0 (0%)
USG	1.015 ± 0.007 (1.002-1.030)	1.017 ± 0.006	1.014 ± 0.007
<1.010, n (%)	14 (22.6%)	4 (6.5%)	10 (16.1%)
1.010-1.020, n (%)	31 (50.0%)	12 (19.3%)	19 (30.7%)
1.021-1.030, n (%)	17 (27.4%)	9 (14.5%)	8 (12.9%)
>1.030, n (%)	0 (0%)	0 (0%)	0 (0%)
USG values for UC _{res} range			
UC 1	1.003 ± 0.001 (1.002-1.005)		
UC 2	1.011 ± 0.005 (1.005-1.019)		
UC 3	1.014 ± 0.005 (1.008-1.023)		
UC 4	1.018 ± 0.004 (1.011-1.025)		
UC 5	1.021 ± 0.003 (1.019-1.024)		
UC 6	1.023 ± 0.004 (1.014-1.030)		
UC 7	N/A		
UC 8	N/A		

UC_{sub} = Urine color values determined by subjects; UC_{res} = Urine color values determined by researchers;

USG = average USG of duplicate measures.

Values are mean ± SD (range), unless otherwise indicated.

Table 6. NATA Indexes of Hydration Status for Urine Color and USG Frequencies (4)

Hydration Status	----	Hydration Biomarker		
		UC _{sub}	UC _{res}	USG
Well-hydrated	Frequency, n	19	17	14
	Percent, %	30.6%	27.4%	22.6%
Minimal Dehydration	Frequency, n	30	31	31
	Percent, %	48.4%	50.0%	50.0%
Significant Dehydration	Frequency, n	13	14	17
	Percent, %	21.0%	22.6%	27.4%

For abbreviations, see legend to Table 5. χ^2 value= 1.3726, p=0.848935

Table 7. ACSM Euhydration Cut-off Frequencies (3)

Hydration Status	----	Hydration Biomarker		
		UC _{sub} ^a	UC _{res} ^b	USG ^c
Euhydration	Frequency, n	49	48	45
	Percent, %	79.0%	77.4%	72.6%
Dehydration	Frequency, n	13	14	17
	Percent, %	21.0%	22.6%	27.4%

For abbreviations, see legend to Table 5. χ^2 value=0.774, p=0.679088

National Athletic Trainers Association urine colors indexes used to indicate euhydration based USG of ≤ 1.020 (urine colors 1-4) and ≥ 1.021 to indicate dehydration (urine colors 5-8) (4).

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CHAPTER 3: Conclusions and Future Directions

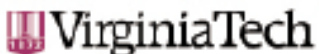
The main findings from our investigation were threefold. First, urine color ratings of both researchers and participants were moderately strong positively associated with USG in NCAA division I college athletes. Second, hydration indices tested were independent of the indexes of hydration status/euhydration cut-off from ACSM and NATA for urine color and USG (1-2). Meaning urine color and USG alone may not identify the appropriate hydration index/euhydration cut-off established by ACSM and NATA. Lastly, participant self-assessed and researcher assessed urine color displayed a strong positive relationship with strong agreement.

There are several avenues for future work that are needed. First, it is possible that our investigation included too few hydration indices to establish a dependent relationship with the NATA and ACSM indexes of hydration/euhydration cut-off in NCAA division I college athletes. Therefore, future research of other hydration indices (i.e. urine osmolality, % body weight changes) should be investigated. Second, the strength of the relationship between urine color and USG should be assessed among individual sports. It is possible that some sports, particularly sports that rely mainly on intentional dehydration practices to meet weight classes, may exhibit correlations between urine color and USG that are different in magnitude. Third, assessing the strength of the relationship between USG and urine color across levels of hydration status would be useful. Prior research has reported a lower correlation between urinary indices (urine color, USG, urine osmolality) in hypohydrated, compared to euhydrated individuals (3-4). Lastly, quantifying fluid intake required to change urine and USG in NCAA division I college athletes would be invaluable.

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2. Casa DJ, Armstrong LE, Hillman SK, et al. National Athletic Trainers' Association position statement: fluid replacement for athletes. *J Athl Train.* 2000;35(2):212-224.
3. Armstrong LE, Maresh CM, Castellani JW, Bergeron MF, Kenefick RW, LaGasse KE, Riebe D. Urinary indices of hydration status. *Int J Sport Nutr.* 1994;4(3):265-279.
4. Fernández-Elías VE, Martínez-Abellá A, María López-Gulló J, Morán-Navarro R, Pallarés JG, Cruz-Sánchez E, Mora-Rodríguez R. Validity of hydration non-invasive indices during the weightcutting and official weigh-in for Olympic combat sports. 2014.

APPENDIX A: Institutional Review Board Approval



Office of Research Compliance
Institutional Review Board
North End Center, Suite 4120, Virginia Tech
300 Turner Street NW
Blacksburg, Virginia 24061
540/231-4606 Fax 540/231-0959
email irb@vt.edu
website <http://www.irb.vt.edu>

MEMORANDUM

DATE: February 3, 2017

TO: Kevin Davy, Michelle S Rockwell, Catherine Whitaker Cockrill, Brittany Ryann Thorpe, Brenda Davy

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)

PROTOCOL TITLE: Validation of Beverage Intake and Hydration Status in Collegiate Athletes 

IRB NUMBER: 17-048

Effective February 2, 2017, the Virginia Tech Institutional Review Board (IRB) Chair, David M Moore, approved the New Application request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at: <http://www.irb.vt.edu/pages/responsibilities.htm>

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: Expedited, under 45 CFR 46.110 category(ies) 3,4,7
Protocol Approval Date: February 2, 2017
Protocol Expiration Date: February 1, 2018
Continuing Review Due Date*: January 18, 2018

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

Date*	OSP Number	Sponsor	Grant Comparison Conducted?

* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.

NCAA DIVISION 1 ATHLETES NEEDED

Did you know beverage intake & hydration can impact your athletic performance?

If you meet the following criteria, you may be eligible to participate in a research study to validate tools for assessing beverage intake and hydration status.

Additional Eligibility requirements:

- Men and women 18+ years old
- Member of a Virginia Tech or Radford University NCAA intercollegiate athletic team and currently practicing and/or competing without restrictions
- Does not have an acute injury or illness

This study will require **3 visits** at your athletic center and a time commitment of **approximately 2-2.5 hours**.

You will receive: a detailed dietary and beverage intake analysis as well as information about your hydration status (if you are adequately hydrated before practice).

If you meet the above requirements and are interesting in participating in this research study, please contact the Human Integrative Physiology Laboratory at Virginia Tech by **email:** bthorpe@vt.edu & catwc94@vt.edu or by **phone:** 540-231-0923.

APPENDIX C: Informed Consent

Consent Document Department of Human Nutrition, Foods, and Exercise Virginia Tech

TITLE: Validation and Measurement of Hydration in College Athletes

INVESTIGATORS: Catherine W. Cockrill; Brittany Thorpe; Brenda M. Davy, PhD, RD; Michelle Rockwell, MS, RD, CSSD; and Kevin Davy, PhD

PURPOSE

The dietary habits and fluid intake of collegiate athletes are an important determinant of both athletic performance and overall health. However, training, travel, and school schedules in addition to financial, emotional and mental stressors associated with the collegiate environment are all barriers to optimal dietary intake. Currently, there are limited resources to accurately assess fluid intake among athletes. In addition, urine color is commonly used to identify an athlete's hydration level, but there is limited information regarding its' validity. The purpose of the present study is to determine: 1) the accuracy of a beverage intake questionnaire called the BEVQ15 and 2) if urine color is an accurate measure of hydration status. Sixty college athletes, aged 18 years and older will be studied.

You will be asked to complete a dietary intake recall three times, a beverage intake questionnaire two times, and provide two pre-practice urine samples over a two-week period.

PROCEDURES

You will be required to attend three in person testing sessions within two weeks in a team meeting room or another location (e.g., media room) in the Dedmon Center for Radford athletes or an athletic facility for Virginia Tech athletes.

Session 1 and 2 (Time Commitment ~ 1 hour)

General Information Questionnaire: You will be asked to complete a questionnaire which includes information about your age, race/ethnicity, sex, year of eligibility, sport, and role on your team (e.g., position, starter, etc). You will only complete this questionnaire one time.

Body Weight/Height: Your weight will be measured on a digital scale and will include the weight of light indoor clothing without your shoes. Your height will be measured using a stadiometer (ruler on the wall).

Dietary intake analysis (24-hour recall): You will be asked you to recall all the food and beverages you consumed in the previous 24-hour period.

Beverage Questionnaire: You will complete a short questionnaire specifically asking about your usual beverage consumption.

Urine Collection: You will be asked to provide a 2-3 teaspoon urine sample in a cup provided. The color of your urine will be assessed using a chart and the urine specific gravity (the amount of particles dissolved in your urine) will also be measured using a handheld device called a refractometer.

Session 3: (Time Commitment ~30 min)

Dietary intake analysis (24-hour recall): You will be asked you to recall all the food and beverages you consumed in the previous 24-hour period.

The total time commitment for this study will be ~2.5 hours.

SUMMARY OF SUBJECT RESPONSIBILITIES

- Be on time and attend all scheduled sessions.
- Follow all participant instructions for each session.

RISKS OF PARTICIPATION

- **Dietary Intake Assessment:** As part of a complete dietary intake assessment you will be asked to report any alcohol you may have consumed. This information is confidential and will not be shared with anyone outside the research team.
- It is not possible to identify all potential risks. However, the staff will take all possible safeguards to minimize any known and potential risks to your well-being. All of the procedures are will established and used routinely in the investigator's research program.

BENEFITS OF PARTICIPATION

Your participation will provide you with:

- A detailed nutritional analysis of your food and beverage consumption
- Information on your hydration status

CONFIDENTIALITY

The data from this study will be kept strictly confidential. No identifiable data will be released to anyone but those working on the project. Subject numbers without anything to identify your name will identify data. De-identified team and overall summary results may be shared with coaches and sports medicine staff of the Virginia Tech and/or Radford athletic departments.

FREEDOM TO WITHDRAW

You are free to withdraw from the study at any time for any reason. Simply inform the researchers of your intention to cease participation. Circumstances may come up that the researcher will determine that you should not continue as a subject in the study. For example,

lack of compliance to instructions, failure to attend testing sessions and illness could be reasons for the researchers to stop your participation in the study.

INJURY DURING PARTICIPATION

Neither the researchers nor the university have money aside to pay for medical treatment that would be necessary if injured as a result of your participation in this study. Any expenses that you incur including emergencies and long-term expenses would be your own responsibility. You should consider this limitation before you consider participating in this study.

APPROVAL OF RESEARCH

This research has been authorized, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Tech. You will receive a copy of this form to take with you.

SUBJECT PERMISSION

I have read the informed consent and I have had all my questions answered to my satisfaction. I hereby give my voluntary consent to be a participant in this research study. I understand that I may withdraw from the study at any time.

If you have any questions, you may contact:

- Kevin Davy, PhD, Professor, Department of Human Nutrition, Foods, and Exercise. (540) 230-0486 or (540) 231-3487.
- Catherine Cockrill, Master’s Student, Department of Human Nutrition, Foods, and Exercise. (571) 271-9308 (cell) OR Brittany Thorpe, Master’s Student, Department of Human Nutrition, Foods, and Exercise. (810) 965-4845 (cell).
- David Moore, Chairman, VT Institutional Review Board: (540) 231-4991

Name of Subject (please print) _____

Signature of Subject _____ **Date** _____

APPENDIX D: Athlete Anthropometric & General Information Data Collection Form

**Virginia Tech
Department of Human Nutrition, Foods, and Exercise**

Athlete Anthropometric & General Information Data Collection Form

SUBJECT ID # _____

DATE _____

TIMEPOINT(1st or 2nd visit): _____

Age: _____

Sex: _____

Sport: _____

Year of Eligibility (Freshmen, Sophomore, etc): _____

How would you classify your current role on the team (Circle 1)?

Starter

Regular Substitute

Sporadic Substitute

Bench/Practice Player

Classification Definitions:

Starter- Starts and plays majority of games/matches.

Regular Substitute- Makes an appearance in >50% of games/matches.

Sporadic Substitute- Makes an appearance in <50% but >5% of games/matches.

Bench/Practice Player- Makes appearances in ≤5% of games/matches or is a (nonmedical) redshirt.

To Be Completed By Research Staff:

Height (cm): _____

Weight (kg): _____

BMI (kg/m²): _____

Research Staff Initials: _____


APPENDIX E: Urine Color Chart


ASSESS YOUR HYDRATION STATUS

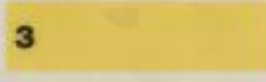
Step 1: Match the color of your urine to a color on the chart.


Step 2: If your urine color matches:

- Nos. 1, 2 or 3, you are hydrated.
- Nos. 4, 5, or 6, you are mildly to moderately dehydrated.
- Nos. 7 or darker, you are dehydrated.

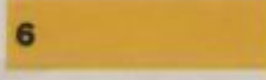
1 

2 

3 

4 

5 



6 


7 

8 

You should know...

- ▶ You should consume water throughout the day. Do not wait until you are thirsty.
- ▶ In general, 20 ounces of fluid should be replaced for every pound of body weight lost during an exercise session.
- ▶ Although not common, certain foods, medicines and vitamins may cause the color of urine to change.
- ▶ For further information visit: www.hydratecheck.com



 **HydrationCheck**

References:

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- Armstrong, L.E., Herrera-Soto, J.A., Heckler, T.T., Casa, D.J., Kavonius, S.A., Weiser, C.M. (1998). "Urinary indices during dehydration, exercise, and rehydration." *Int. J. Sport Nutr.*, 8: 345-355.

APPENDIX F: Urine Collection Data Sheet

Subject ID _____

UC visit # _____, Researcher _____

I believe my urine color is # _____.

Subject ID _____

UC visit # _____, Researcher _____

Subject's urine color is # _____.

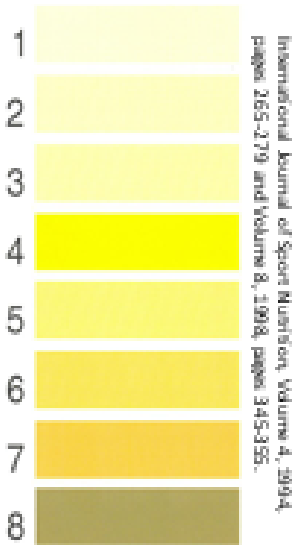
USG: 1) _____ 2) _____

APPENDIX H: Hydration Values Report

ATHLETE NAME: _____

HYDRATION VALUES

Are you Hydrated Enough Before Practice?



If your urine color is:

- **1-3** you are **WELL-HYDRATED.**
- **4-6** you are **DEHYDRATED**
- **7-8** you are **SEVERELY DEHYDRATED**

Your Urine
Color #'s:

Visit#1: _____

Visit#2: _____

Another indication of hydration status is urinary specific gravity (USG).

USG looks at how concentrated the urine is. Urine that is less concentrated indicates more hydrated values and more concentrated values indicate more dehydrated values.

If your urine USG is:

- **1.001-1.020** you are **WELL-HYDRATED.**
- **1.021-1.030** you are **DEHYDRATED**
- **>1.030** you are **SEVERELY DEHYDRATED**

Your USG #'s:

Visit#1: _____

Visit#2: _____

Fluid Recommendations

Minimum: 3.7 Liters/day for men, 2.7 L/day for women OR 1 ml fluid per calorie consumed.

Before Exercise: in the 4 hrs before practice you need 5-7 ml fluid/kg body weight OR 400-600 mL fluid

During Exercise: consume 7-10 fluid ounces (~ 200-300 mL fluid) every 10-20 minutes

After Exercise: Replace fluid lost from sweat, by weighing yourself before and after practice. Drink 16-24 oz fluid for each lb lost OR 1-1.5 L of fluid for each kg of weight lost.