

The Effect of Instrument Type on the Measure of Hydration Status

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ABSTRACT

Context: Although some instruments have been validated for clinical measure of hydration status, new and currently invalid instruments are available for purchase and clinical use. Athletic trainers commonly use these instruments to assess hydration status for weight checks and body mass loss charts due to their ease of use. However, the validity of these popular instruments has not yet been established. **Objective:** To determine the validity of urine specific gravity (USG) for the assessment of hydration status via the following instruments: handheld clinical refractometer, pen style digital refractometer, and midjet urinometer as compared to the gold standard urine osmometer (OSMO). **Design:** Descriptive diagnostic validity study. Setting: Biochemical research laboratory. **Patients or Other Participants:** Healthy active men and women (n=108; mean age=22±4yrs; self reported height=174±20cm and mass=75±17kg) were recruited among faculty and students on a university campus. Interventions: The independent variable was instrument type with four levels: osmometer, handheld clinical refractometer, pen style digital refractometer, and midjet urinometer. After recruitment, participants completed an informed consent and a short health history questionnaire to rule out any exclusionary criteria such as kidney disease or chronic urinary tract infection. Participants were then given a clean standard urine cup and asked to provide as much sample as possible, providing more than one cup when possible. **Main Outcome**

Measures: Hydration status was measured by USG and OSM. USG was evaluated by a handheld clinical refractometer, pen style digital refractometer, and midjet urinometer. The gold standard OSM was calculated by a freezing point depression osmometer. Z scores were calculated for each instrument and Pearson product-moment correlation coefficients were evaluated to examine the relationship between each instrument of USG and OSM. **Results:** Strong significant correlations were identified for the digital refractometer ($r=0.814$, $p < 0.001$) and handheld clinical refractometer ($r=0.943$, $p < 0.001$) with OSM. A weak statistically insignificant correlation was established between the midjet urinometer ($r=0.133$, $p < 0.142$) and OSM. Average hydration status indicated variability among some of the instruments: digital refractometer USG= 1.0194 ± 0.0075 , clinical refractometer USG= 1.020 ± 0.007 , urinometer USG= 1.028 ± 0.091 , osmometer OSM= 743 ± 271) **Conclusions:** Handheld clinical refractometry can be used confidently for assessing hydration status as it shows a strong significant correlation with the gold standard osmometer, which is consistent with previous literature. Additionally, the use of the pen style digital refractometer showed a strong, significant correlation with the gold standard osmometer and provides clinicians with another option for the clinical assessment of USG and hydration status. The findings of this also study suggest that the use of a midjet urinometer should be performed with extreme caution, as it showed a weak correlation with the gold standard osmometer, indicating it might not provide accurate results when used to determine hydration status.

PREFACE

As with many ambitious graduate students, I wanted to develop a project that would have clinical applicability and make an impact on the way athletic trainers practiced. When I first began my journey in graduate school I was unsure of what direction to take my research. There were so many captivating topics to explore and questions I wished to answer. With the help of my committee and some close peers, we managed to create a study that we felt would be important for the practicing clinician as well as the inquisitive researcher. As I look back on all of the hard work and time put into this project, I feel a sense of pride and accomplishment. Some of my most fond memories of graduate school will take me back to stories and conversations shared while working on this project.

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CHAPTER 1

INTRODUCTION

Proper levels of hydration are important for normal physiological function of all body systems.^{1,2} Deviations from a euhydrated state may occur for a variety of reasons and the process by which the body loses water, dehydration, occurs in a variety of populations. For example, research has identified that youth athletes are hypohydrated during camps and practices.³⁻⁵ Similarly, research on the adult population has revealed that a significant percentage (53%) arrive to preseason practices hypohydrated.⁶ Hypohydration is a risk factor for heat related illnesses including exertional heat stroke.^{1,2,7} Hypohydration causes detrimental physiological changes that predispose individuals to heat illnesses. These physiological changes include: increased heart rate, decreased cardiac output, increased physiological strain, increased perceived strain, decreased muscular power, and decreased cognitive performance.^{1,2,8} These factors alter thermoregulation in the body therefore, increasing the body's susceptibility to heat illness.^{1,2,7}

Many methods exist for hydration status measurement; however only a few methods are valid and reliable for clinical measurements. The urinary indices, namely urine specific gravity and urine osmolality, are two of the most common methods for determining hydration status. These methods are practical and easy to use for clinicians and researchers. The osmometer is

used to measure urine osmolality and works by comparing the freezing point of the urine specimen to the freezing point of water.^{9,10} Urine osmolality is considered the urinary “gold standard” for measuring hydration because of its ability to measure solutes in concentration.^{9,11,12}

Urine specific gravity is defined as the ratio of the densities between urine and water and is therefore determined by the number of particles in concentration of a sample.^{9,10,13} Specific gravity is known as the most practical and cost effective means of measuring hydration status.⁹ Urine specific gravity can be assessed with several tools: clinical refractometer, digital refractometer, urinometer, and reagent strips. A clinical refractometer works by viewing fluid under normal light and detecting the amount of particles in the fluid.⁹ The same principles are true with a digital refractometer; however, the instrument determines the particles in the fluid without viewing. Assessing hydration status with a urinometer is another approach for measuring specific gravity, using Archimedes’s principle. Reagent strips, although still common amongst clinicians have been refuted in the literature by several studies. Research is lacking regarding the use of the digital refractometer and urinometer.

Based on research and findings the American College of Sports Medicine (ACSM), National Athletic Trainer’s Association (NATA,) the National Collegiate Athletic Association (NCAA) and National Wrestling Coaches Association (NWCA) have given recommendations for hydration testing.^{1, 2, 14} These organizations provide support for many different tools for assessing hydration status. The variety in methods of assessing hydration status in literature may be confusing for clinicians. These inconsistencies may cause issues in reaching proper outcomes for the measure of hydration status. Therefore, the purpose of this study is to determine the

validity of urine specific gravity via clinical refractometer, digital refractometer, and urinometer as compared to urine osmolality.

Research Question

Which of the following instruments are comparable to the gold standard osmometer in determining hydration status: clinical refractometer, digital refractometer, and urinometer?

Hypotheses

1. The clinical refractometer will strongly correlate with the osmometer when examining hydration status.
2. The digital refractometer will strongly correlate with the osmometer when examining hydration status.
3. The urinometer will have a moderate correlation with the osmometer when examining hydration status..

CHAPTER 2

REVIEW OF LITERATURE

Proper levels of body hydration are important for proper physiological function and performance. Alterations in hydration may occur for a variety of reasons including physical exertion, environmental conditions, and illness. Understanding changes in hydration levels is important for health care practitioners and researchers working with populations exposed to exercise in these conditions. This review of literature describes how altered hydration affects physiology and performance in different environments, illnesses and during exertion. Additionally, this review of literature will examine existing methods for assessing hydration status that health care providers and researchers utilize.

Search Strategy

Searches on the topic were completed in the following databases: PubMed, PubMed Central, CINAHL, EBSCOhost, Medline, and hand searching. The following terms were used individually or in combination to search the literature: hydration, hydration status, hydration assessment, heat, cold, altitude, hypohydration, dehydration, diabetes, osmolality, hydrometry, urine specific gravity, refractometry, urine conductivity, urinometer, thirst, urine output, fluid replacement, plasma volume shift, military, marathon runners, and athletes. Exclusion criteria included unhealthy populations and animals.

Definition of Hydration

The body is comprised primarily of water, approximately 73% of the body's lean mass.² Body water is distributed amongst the body's cells and plasma, at rest approximately 30% to 35% of body water is intracellular fluid, 20% to 25% is interstitial fluid, and 5% is plasma.² Total body water balance between spaces and tissues, or euhydration, is important for the normal physiological function of all body systems and is considered the ideal state of hydration.^{1,2} Deviations from this euhydrated state may occur for a variety of reasons.

The state of being less than euhydrated is referred to as hypohydration, whereas the process of becoming hypohydrated is referred to as dehydration.¹ In a hypohydrated state the body has lost body water greater than 1% of body mass.¹ According to the American College of Sports Medicine position stand on exercise and fluid replacement, a person may be defined as euhydrated if their first morning void is $USG \leq 1.020$ or $UOsmol \leq 700 \text{ UOsmol} \cdot \text{Kg}^{-1}$.¹

Hypohydration and Physiological Alterations

Total body water balance is necessary for normal physiological function.² During physical exertion, individuals are subjected to various environmental conditions and workloads causing them to sweat. Sweat is a hypotonic solution to body water.² Due to changes in hydrostatic pressure and osmotic-oncotic gradients when sweating, water moves from intracellular to extracellular spaces.² Losses of body water result in an overall hypovolemic-hyperosmolality state in the body.² This state is considered to be the catalyst for the physiological changes associated with hypohydration.²

Physiology and Thermoregulation

Hypohydration has a significant impact on the body's ability to thermoregulate in the heat.² Compromise of the body's thermoregulatory system occurs due to increased

cardiovascular strain.^{2,15} Increased cardiovascular strain is a product of decreased stroke volume, increased heart rate, increased systemic vascular resistance, decreased mean arterial pressure, and decreased cardiac output.^{2,15} Cardiovascular strain arises from decreased blood volume and impairs the body's ability to promote skin blood flow for cooling and sweat responses.²

Increased cardiovascular strain causes excessive heat production and heat storage in the body.^{2,15} Essentially, the body has an inadequate volume of blood (due to fluid loss) to send to the skin for cooling (conductive and convective) and maintain the required cardiovascular needs of working tissues. Consequently, for every 1% body mass lost during exercise, core body temperature increases .15 to .20°C, and heart rate three to five beats per minute.^{2,8}

Exercise Performance

Hypohydration and subsequent altered thermoregulation have significant implications on exercise and sport performance. The degree of hypohydration dictates the severity of overall physiological compromise.² A hypohydrated state of 2% dehydration or greater can decrease aerobic performance, increase physiological strain, perceived strain, and decrease cognitive performance.^{1,2,8} Muscular endurance and strength can be affected at 3-5% dehydration.² The performance decrements that occur with 2.5% dehydration and greater occur regardless of fitness level and acclimatization.² In summary, hypohydration leads to decreased endurance performance, decreases time to exhaustion, and increases heat storage in the body.^{7,16}

Hypohydration and Heat Illnesses

Exertional Heat Stroke

Exertional heat stroke is defined by a core body temperature greater than 40°C and is associated with organ system failure, and central nervous system depression.^{7,16} Exertional heat stroke occurs when the body's thermoregulatory system is unable to properly manage and

dissipate heat.^{7,16} When dehydration of 3%-5% body weight occur, cooling mechanisms such as skin blood flow and sweat production begin to decline, thereby decreasing the body's ability to dissipate heat.⁷ Therefore, hypohydration is considered a risk factor for heat stroke.^{1,2,7,16}

Exercise Associated Muscular (Heat) Cramps

Exercise associated muscular cramps (EAMCs) are short term, painful, involuntary spasms of skeletal muscles that occur during or after prolonged, intense exercise, usually in the heat.^{7,16} EAMCs commonly occur in the legs, arms and abdomen.⁷ Sodium imbalance as a result of sweating is considered an underlying physiological cause of EAMCs.⁷ Therefore, sweat induced dehydration and fluid-electrolyte imbalances from sweat Na⁺ losses are an accepted risk factor for EAMCs.^{7,16} Other hypothesized causes include neuromuscular fatigue, genetic metabolic abnormalities, as well as these factors in combination.^{7,16}

Hypohydration and Cold Exposure

Physiology

Exposure to cold and dry air causes the body to make physiological adaptations, namely, peripheral vasoconstriction and air humidification.¹⁷ Peripheral vasoconstriction occurs in the extremities when the central nervous system senses decreased skin temperature usually between 34°C – 35 °C.¹⁷ The goal of the peripheral vasoconstriction is to decrease the amount of warm blood being sent to the extremities, consequently decreasing the amount of body heat that will be lost to the cold ambient to maintain an core body temperature.¹⁷ By decreasing the amount of blood circulating to the extremities, an increased amount of warm blood stays in the core, increasing the central blood volume.¹⁷ The maintenance of central blood volume alters blood pressure and stimulates baroreceptors eventually stimulating a physiological process called diuresis. Diuresis is a function of the kidneys and causes the body to excrete increased blood

volume at the core in the form of urine.¹⁷ When cold induced diuresis occurs over an extended period of time, it causes excess urine output and eventually dehydration.¹⁷ Dehydration via cold-induced diuresis decreases the body's total blood volume as well as plasma volume.¹⁸

Dehydration can also occur due to the humidification and warming of the cold dry air during ventilation.¹⁷ Research has shown that in 0°C air water loss can be up to .9L per day and in -20°C air up to 1L per day can be lost from the humidification of air.¹⁸ The quality of exercise performance in cold environments has been shown to be dependent upon the degree of hypohydration, as well as the intensity, frequency and duration of exercise being performed.¹⁸ Muscular power has been found to be affected by internal muscular temperature.¹⁸ As internal muscle temperature decreases, muscular power output decreases as a result of the decreased speed of ATP synthesis.¹⁸

Hypothermia and Frostbite

Hypothermia generally refers to when the body experiences a decreased core temperature. There are varying severities of hypothermia, the cooler the core body temperature the more severe the hypothermia. Prolonged exposure to cool (50 °F or less), wet, windy environmental conditions increases the likelihood of experiencing hypothermia.¹⁷ When spending time in cold conditions, the body generates heat to maintain a homeostatic core body temperature in two ways: metabolic heat production and shivering. Shivering is the primary mechanism the body uses to generate heat.¹⁷ Shivering intensity is determined by the severity and duration of cold exposure and generally occurs in the large muscles of the trunk first.¹⁷ The body strives to conserve adequate levels of heat in cold conditions. Heat conservation is a

product of peripheral vasoconstriction. As mentioned previously, peripheral vasoconstriction decreases the amount of warm blood that is circulating from the core to the cooler extremities.

Frostbite occurs when there is actual freezing of body tissue.¹⁷ Just as in hypothermia, there are varying levels of severity of frostbite, the deeper and more extensive the tissue damage, the more severe the frostbite. Frostbite occurs due to the body's protective peripheral vasoconstriction mechanism. The furthest extremities (toes, nose, fingers, etc) are the most sensitive areas to local temperature and blood vessel constriction.¹⁷ Distant extremities are not able to sense if the body's core temperature is adequate.¹⁷ Consequently, even if the core is at an adequate temperature, the blood vessels that supply cold extremities continue to redirect blood to the core.¹⁷ This absence of warm blood leads to extensive temperature loss in the extremities eventually freezes the tissue.

As mentioned previously, dehydration can occur secondarily to peripheral vasoconstriction, however current research has shown that dehydration does not affect the body's ability to produce and conserve heat through shivering and peripheral vasoconstriction.¹⁷ Essentially, to the body, maintaining core temperature is more important than maintaining fluid balance. Therefore, dehydration is not necessarily a risk factor for hypothermia and frostbite, but more of a symptom of cold exposure.

Hypohydration and Altitude Exposure

Physiology

Ascending to high altitude is commonly associated with moderate to severe dehydration.¹⁹ As individuals ascend to high altitude, the partial pressure of oxygen decreases, the humidity of air decreases, and the temperature of the air decreases.^{18,19} In an effort to counteract decreased oxygen saturation in the blood, the rate of ventilation increases.^{18,19}

Increased ventilation of cold dry air causes increased ventilatory water loss.^{19,20} On average, 2-1.5L can be lost per day, depending on resting ventilation and increases in ventilation associated with exercise.^{19,20} In extreme cases, as much as 7L per day can be lost due to high altitude exposure.¹⁹ Additionally, diuresis occurs due to changes in atmospheric pressure.²¹ Altitude associated diuresis causes increases in the hemoconcentration of circulating blood in an effort to counteract the decreased partial pressure of oxygen.²¹

Performance is impaired at altitude due to decreased max heart rate, decreased arterial oxygen saturation, decreased cardiac output, decreased VO₂ max, and increased lactic acid accumulation.^{18,19} Dehydration that occurs due to altitude exposure (and subsequent cold exposure) leads to an increased blood viscosity that also additively contributes to the decrease in the oxygen carrying capacity of blood.¹⁹

Hypohydration and Altitude Related Illnesses

General dehydration that occurs from altitude exposure, as well as hypoxia and decreased hemoconcentration, are considered as possible risk factors for high altitude illnesses.^{19,22} Research suggests that consuming less than 3000mL of fluid per day can increase the risk of acute mountain sickness (AMS) by 60%.^{19,23} Subsequent recommendations for the prevention of AMS state that individuals at high altitude should consume at least 5-7L of fluid per day in order to counteract cold-altitude related dehydration.^{19,23} Laboratory simulated high altitude exposure investigations by Richardson et al. discovered that a hypohydrated state has detrimental effects on exercise performance and AMS symptoms (Lake Louise questionnaire, headache assessment, and environmental symptoms questionnaire).²³ Subsequent laboratory investigation by Richardson et al found that 2% hypohydration in a hypoxic environment increases

physiological strain.²⁴ Additionally, Richardson et al. discovered that as dehydration increases incrementally, so does the severity in measures of AMS via the Lake Louise questionnaire.²⁴ Likewise, a field study at sea level and high altitude by Castellani et al. found the combination of hypohydration and altitude exposure to have more detrimental effects on exercise performance as compared to exercise performance at sea level.²⁵ Castellani et al. also revealed that the combination of hypohydration and high altitude have more significant impact on exercise performance than either condition independently.²⁵ However, the Castellani study found hypohydration did not correlate with symptoms of AMS.²⁵

Hypohydration and Diabetes

Patients with poorly managed glucose levels are at higher risk for hyperglycemia and ketoacidosis, which cause dehydration.^{26,27} When blood becomes hyperglycemic, there is an increase in the osmolality, which triggers osmotic diuresis.²⁷ Diuresis triggers increased rates of urination. In the diuretic induced urinary excretions are increased amounts of free water, excess glucose, and electrolytes.²⁷ The over excretion of glucose and electrolytes contributes to acid-base imbalance and ketoacidosis.²⁷ Diabetic ketoacidosis is a medical emergency and clinical presentation is generally comprised of hyperglycemia, acidosis, and weight loss via dehydration (up to 6L total body water).²⁷ Emergency treatment of diabetic ketoacidosis is focused primarily on intravenous rehydration, electrolyte replacement and insulin therapy to restore acid-base imbalance.²⁷

Prevalence of Hypohydration

Adolescents

Hypohydration occurs in a variety of populations. Youth athletes are dehydrated during camps and practices.³⁻⁵ Decher et al. found that in a sample of approximately 70 adolescent boys

and girls average hypohydration ranged from minimal to severe across 4 days.³ Likewise, McDermott et al. found that in a sample of 33 adolescent boys at a 5 day football camp were hypohydrated.⁴ Yeargin et al. found that high school football players replaced their sweat losses during practice but were still mildly hypohydrated for the duration of the 10 day preseason football practice data collection.⁵

Adults

Similar trends exist in the adult athlete population. Overall from 2005 – 2009 118 cases of heat illness that caused loss of participation time, defined as dehydration, heat exhaustion, or heat stroke were reported.²⁸ Athletes tend to arrive to summer workouts and pre participation examinations hypohydrated.^{29,30} In a sample of 288 football players across varying levels, National Collegiate Athletic Association (NCAA) Division III to National Football League (NFL), approximately 45% were moderately dehydrated and 15% were significantly dehydrated. Yeargin et al. found that in a sample of 403 athletes from various collegiate sports and ability levels that approximately 53% were hypohydrated.⁶ Likewise, Volpe et al. found that in a sample of 263 NCAA men and women athletes, 15% were significantly hypohydrated and 53% were moderately hypohydrated.³¹ The same study found that 47% of the males were hypohydrated whereas only 28% of the 125 females were hypohydrated.³¹

This phenomenon is not confined simply to football and collegiate athletes. Osterberg, Horswill, and Baker examined 29 professional basketball players from various National Basketball Association (NBA) teams and found that approximately half were hypohydrated before games.³² Stover et al. examined the hydration status of recreational athletes before exercise and found that 46 % of the men and women participating were hypohydrated.³³

Consistent with other studies that have examined both men and women, Stover et al. found men to be more dehydrated than women.³³ An examination on the pre and post work shift hydration status of forestry workers in two different seasons of the year found that in the fall 43% of 103 participants were hypohydrated and that 47% of the 79 participants were hypohydrated in the winter.³⁴ Gardener et al. has described dehydration and its relationship with exertional heat illness as a significant cause of morbidity and mortality in United States military recruits training in the heat.³⁵ Investigation by Laursen et al. showed that on average Iron Man triathlon competitors became more dehydrated during competition.³⁶

Methods of Assessing Hydration

Hematological Analysis

Plasma, or the fluid portion of blood, comprises approximately 5% of body mass.¹⁰ Dill and Costill state that when a person is severely dehydrated the volume of plasma will decrease.³⁷ Therefore, when an individual sweats, it is postulated that the fluid portion of sweat is a product of plasma and extracellular fluid.³⁸ The concentration of plasma in blood, or plasma volume can be determined by assessing hematocrit and hemoglobin concentration of a blood sample.¹⁰ Classic investigation by Dill and Costill found changes in plasma volume can be used to properly assess dehydration.³⁷ A simple equation using the plasma volumes (PV) obtained from the hematocrit before (PV_B) and after (PV_A) are used to determine the plasma volume change $[(\Delta PV, \% = 100 (PV_A - PV_B)/PV_B)]$.³⁷ This equation has been historically popular due to its ease of use, cited in over 1300 peer reviewed scientific publications between 1994-2004.¹⁰ Despite the popularity of plasma volume shift analysis, there are some limitations: training to take venous blood samples is required, there is risk for infection, and possibility of vessel damage.³⁹ Therefore, the use of plasma volume shifts is not always the best choice. Additionally taking

blood samples and using plasma volume shift is not practical for practicing athletic trainers in the field.

Plasma osmolality is a common hematological analysis used by researchers and is considered by some to be the only valid measure of hydration status.¹⁰ Plasma osmolality is based on plasma volume shifts and extracellular fluid.³⁸ When an individual sweats plasma and extracellular fluids decrease in concentration changing the osmolality of the blood.³⁸ When used in conjunction with total body water assessment some consider plasma osmolality the “gold standard” for hydration assessment.⁴⁰ Oppliger et al. found plasma osmolality to be more sensitive to incremental changes in dehydration based on percent body weight loss during exercise as compared to urine specific gravity and urine osmolality.¹¹ Plasma osmolality is calculated with the use of either a freezing point or vapor pressure depression osmometer.¹⁰ Plasma osmolality is considered beneficial and accurate, but is complicated, complex and requires extensive training for use and obtaining samples.^{10,38,40,41}

Total Body Water/Doubly Labeled Water

Doubly labeled water is a method of assessing hydration status, a known amount of non radioactive isotope, commonly $^2\text{H}_2\text{O}$, is consumed.³⁸ A sample of a body fluid is then draw and concentration of the isotope is determined.³⁸ Once the concentration of the isotope is determined, the total body water can be determined. A low concentration of isotope would indicate a greater amount of total body water (diluted isotope in body water) and subsequently appropriate hydration.³⁸ Isotope dilution has been found to be reliable between days and accurate.^{38 10,40} This method of hydration assessment is also considered an appropriate laboratory

measure but, due to its complicated and complex nature, not practical for the practicing athletic trainer (AT).^{38,40}

Urinary Indices

Urine osmolality is the amount of particles in a solution.^{9,10} Armstrong et al. described urine osmolality as being more accurate than other urinary indices of hydration because it is not affected by solutes such as glucose, protein, and urea that may be in the urine sample.⁴² An osmometer is used to measure osmolality and works by comparing the freezing point of the specimen to the freezing point of water.^{9,10} Essentially, the more solutes dissolved in the specimen, the lower the freezing temperature of the specimen in comparison to the freezing point of water.^{9,10} Urine osmolality is considered the urinary “gold standard” for measuring hydration because of its ability to measure solutes in concentration.^{9,11,12}

Various references for osmolality values of euhydration have been reported. Armstrong et al. stated that a euhydrated value from an initial morning sample should be between 805-867mOsm/kg, whereas Oppliger et al. states values less than or equal to 90mOsm/L may represent euhydration.^{10,39} Investigation by Popowski et al. found that urine osmolality had a nonsignificant statistical correlation of ($r = .43$) with plasma osmolality.⁴¹ In this same study Popowski et al. also found that urine osmolality was sensitive to incremental changes in dehydration but not rehydration when large volumes of fluid were ingested quickly.^{39,41} However beneficial, accurate and appropriate for researchers, urine osmolality is expensive and requires technical training and is therefore not practical for the practicing AT.⁹

Urine specific gravity is defined as the ratio of the densities between urine and water and is determined by the number of particles in concentration.^{9,10,13} Specific gravity has been

suggested as a practical and cost effective method of measuring hydration status.⁹ Specific gravity is an easy, non invasive, convenient method of measuring hydration.⁹ The range of measure is from 1.002 μ G to 1.030 μ G.^{9,10,13} Values between 1.010 μ G and 1.020 μ G are considered minimal dehydration, and values above 1.020 μ G are considered severe dehydration.^{9,10,13} Urine specific gravity can be measured with a variety of instruments.

Clinical refractometry is a common method of obtaining urine specific gravity measures. Clinical refractometry works by viewing fluid under normal light and detecting the amount of particles in the fluid.⁹ Clinical refractometry has been found to have a strong correlation with urine osmolality ($r = .87$), ($r = .87$).^{9,42} Investigation into the relationship between urine osmolality and refractometry by Costa et al. yielded a strong correlation ($r = .81$) as well.⁴³ Refractometry can also be done with a digital refractometer. Unfortunately, no research has been done to validate this technique of refractometry.

Utilizing a urinometer is another approach for measuring specific gravity. The urinometer is based on Archimedes' principle based on fluid density and displacement. Essentially, the lower the density of the fluid, the deeper the object will sink in the fluid. Urinary measures of specific gravity can be obtained using a urinometer by placing a urine specimen into a graduated cylinder and placing a weighted shot ballast into the urine specimen.⁴⁴ Once the shot ballast has sunk and displaced the urine, a urine specific gravity reading from the labeled tip at the top of the ballast can be recorded.⁴⁴ When using the urinometer, the temperature of the sample must be between 20 °C and 22.2°C in order to ensure accuracy.⁴⁴ Investigations into the relationship between the urinometer and osmometer have shown moderate correlation ($r = .60$)⁴⁵

Chemical reagent strips have been used to determine urine specific gravity by simply being placed in a sample. Reagent strips measure urine specific gravity by detecting the amount of H^+ ions in the urine sample and its pH.³⁹ The reagent strip changes in color according to H^+ levels and pH.³⁹ The reagent strip kit includes a color chart that correlates color shades with increments of specific gravity.³⁹ Reagent strips have been shown to have at best, a moderate correlation ($r = .647$, $r = .573$) with urine osmolality.^{9,46} Stuemple and Drury found that reagent strips provided inconsistent measures between testers and trials while providing 15% false negatives for euhydration, 5% false positives for hypohydration and reporting more severe dehydration than refractometry.⁴⁶

Urine color is another viable means of assessing hydration status. Urine color is assessed using a urine color chart numbered according to shade. Number one is the lightest shade and number eight is the darkest shade indicating severe dehydration.^{10,42} Armstrong et al found that urine color had a strong correlation with urine osmolality ($r = .82$) and specific gravity via refractometer ($r = .80$).⁴² Armstrong suggested that urine color was adequate in daily self-hydration measurement and field research settings despite low precision and would therefore be a practical measure for ATs.⁴²

Twenty-four hour urine volume measures the daily flow rate and total urine volume output.¹⁰ Normal urine output for adult males is $1.36 \pm .44$ L per day and $1.13 \pm .43$ L per day with minimum outputs .29L per day and .48L per day respectively.¹⁰ For children between the ages of 10 and 14yrs significantly less output is expected. Normal ranges for boys are $.61 \pm .30$ L per day, girls $.44 \pm .31$ L per day.¹⁰ This method of hydration assessment can be practical if there is cooperation from patients and participants and samples are appropriately obtained.³⁹

Urine conductivity works by measuring the electrical impedance of a urine sample. The electrical impedance is sensed similarly to the way that urine osmolality detects the amount of solutes (Na^+) in the sample.^{39,47} Sherrifs and Maughn have attempted to validate this method of assessing hydration status.³⁹ Conductivity via the Sparta 5 conductance meter has been found to correlate well with urine osmolality when examining the first void of the morning, but questions arise as to its effectiveness immediately post exercise.^{10,47,48} The use of the conductance meter requires a fair amount of training but does provide immediate feedback.^{39,47}

Other Methods of Assessing Hydration Status

Increased perceptual ratings of thirst can approximate the beginning stages of hypohydration at 1-2% of total body water loss.¹⁰ Perceptual ratings of thirst can be measured with a simple numerical scale that rates between 1 (not very thirsty) and 9 (very very thirsty).¹⁰ Ratings between 3 (a little thirsty) and 5 (moderately thirsty) can be presumed to indicate mild dehydration.¹⁰ However, the absence of thirst does not always indicate euhydration.³⁸ Many different variables can affect the ratings of thirst such as: fluid taste, time for consumption, gastric distension, old age, gender, and acclimatization status.¹⁰

Body mass difference is a simple, time efficient method of measuring hydration status. When an individual's caloric expenditure approximately matches intake, a loss of body mass can be attributed to the amount of water lost.¹⁰ Chevront et al. found that body mass change can be a reliable assessment of hydration status as long as athletes have a proper 3 day baseline body mass.⁴⁹ However, a proper euhydrated baseline body mass is difficult to obtain because a significant amount of athletes arrive to practice, workouts, and preparticipation physical exams in a hypohydrated state.^{6,10,29-32,50}

Cheuvront and Sawka devised an easy to use multifactor memory mnemonic device called “W.U.T.” for athletes and clinicians to use to determine hydration status.³⁸ W.U.T. stands for “weight” referring to maintaining a stable body weight and monitoring losses from exertion and sweating, “urine” referring to frequency and color of urine and “thirst” meaning that the presence of thirst may indicate hypohydration.³⁸ The combination of the information obtained from these three parts is recommended to approximate hydration status.³⁸

Recommendations for Hydration Assessment

The ACSM position statement on exercise and fluid replacement recommends the use of the following: Daily body mass change, urine specific gravity or osmolality from the first void of the day.¹ The position stand also states that total body water change is reliable, but unfortunately too impractical for clinical use.¹ Likewise, the NATA position statement recommends using USG via clinical refractometer, urine color, and percent change in body mass for measuring hydration status.²

The NCAA and NWCA policy on weight management requires that all wrestling athletes undergo hydration testing as part of the required weight management program.¹⁴ In order to pass the hydration test and weigh in, athletes must have a urine specific gravity measure of 1.020 or less via refractometer or urinometer.¹⁴ USA track and field provides an advisory paper that advocates personalized fluid replacement for distance runners based on equation-calculated sweat rates using body weight change and urine color.⁵¹

The U.S. military designates specific fluid replacement guidelines for training in the heat lieu of hydration testing. U.S. military fluid replacement guidelines are based on environmental temperature, workload classification, and maximum/minimum totals for hourly fluid consumption in order to match sweat losses.⁵² Research by Kolka et al has found the fluid

replacement guidelines to provide an appropriate method for maintenance of body weight and serum sodium levels in military personnel.⁵²

Conclusion

Research has demonstrated the importance of hydration in preventing illness and maintaining performance. Without a standardized tool for the clinical measurement of hydration status, clinicians may be confused about how to best meet governing body recommendations/requirements. The purpose of this study is to determine the validity of urine specific gravity via clinical refractometer, digital refractometer, and urinometer as compared to urine osmolality

CHAPTER 3

METHODS

Design Statement

This study was a descriptive diagnostic validity test design. The criterion measure was urine osmolality as measured by an osmometer. The dependent measure was USG measured by the urinometer, clinical refractometer, and digital refractometer. Concurrent validity (also known as criterion validity) of each method of USG measurement as compared to the gold standard urine osmolality was assessed.

Participants

We collected 127 samples, from both males and females, between the ages of 18 and 60 years from Indiana State University's campus. No specific inclusion or exclusion criteria were outlined for participation in this study. Indiana State University institutional review board approved the study and participants provided written informed consent to participate in the study.

Measurements and Instrumentation

Urine Specific Gravity

A Fischer brand urinometer, with a USG measurement range of 1.000-1.040 and .001 increments was used. A room temperature (20°C-22.2°C) urine sample (20ml) was poured from a clean urine sample container into a labeled graduated cylinder.⁴⁴ A weighted shot ballast was placed into the sample. As the ballast was released into the sample, it was gently spun and not allowed to touch the sides of the graduated cylinder.⁴⁴ The shot ballast sank, displacing the urine

around it and the specific gravity was recorded from the area where a meniscus formed around the stem of the ballast.⁴⁴ Urinometry shows moderate correlation with osmometry ($r = .60$).⁴⁵

A handheld clinical refractometer (Model A300CL; ATAGO Inc., Bellevue, WA) with a range of 1.000 – 1.060 was calibrated with distilled water. In order to obtain measurements, a small sample of urine was placed on the clear daylight plate of the refractometer via transfer pipette and urine specific gravity measures were recorded to the nearest thousandth. Clinical refractometry is found to be valid, showing a strong correlation with urine osmolality ($r = .87$) and moderate correlation with the urinometer.^{9,43,45,46}

Additionally, an Atago digital hand-held pen refractometer with a range of 1.000 – 1.060 was used to measure urine specific gravity. The tip of the pen refractometer was placed directly into the urine sample cup and the urine specific gravity measure was recorded. To our knowledge there is no research on the validity of this method of measuring urine specific gravity.

Osmolality

Osmolality was measured via osmometer (Advanced Micro – Osmometer Model 3320; Advanced Instruments Inc, Norwood, MA). The osmometer was calibrated before each data collection session, as needed, and according to manufacturer's instructions using known calibration standards. Osmometer range was 0-2000 mOsm/kg H₂O. In order to obtain osmolality measures, approximately 20 μ L of bubble free sample was extracted via osmolality sampler. Once the sample was collected the sampler was cleaned free of any clinging droplets and then placed into the sample port within the operating cradle. The operating cradle was pushed forward and the test was initiated. The osmolality (OSM) of the sample was recorded

from the digital display. Measurements of osmolality were performed twice per sample. If sample values were greater than 5 mOsm/kg H₂O apart, we performed a third test.

Procedures

Participants provided informed consent and completed a health questionnaire (self report height, weight, gender, void of the day, and presence of any of the following: diabetes, chronic urinary tract infection, menstruation, kidney disease, or the use of supplements or vitamins). Upon completing the health questionnaire, participants were administered a clean urine specimen cup and asked to proceed to the restroom to provide as much urine as possible. Hydration status was assessed within two hours of sample collection. In order to reduce the risk of contamination, new osmolality tips and transfer pipettes were used for each hydration assessment. Additionally, proper sanitization of each instrument occurred after each measurement. At the end of data collection urine samples were properly disposed.

Using a transfer pipette, a small sample of urine was taken from the sample cup and placed onto the clinical refractometer, viewed and USG was recorded. The digital refractometer was placed into the sample cup to assess USG and the measure was recorded. After performing assessment with the refractometers, we poured 20mL of urine will be into the graduated cylinder to assess USG with the urinometer. The measure of USG as recorded from the shot ballast piece of the urinometer. Lastly, using a clean osmometer sample tip, we extracted approximately 20 mL of urine and placed it into the operating cradle. Osmolality was recorded from the digital display of the osmometer upon completion of the test. Measures were assessed and recorded by four investigators.

Statistical Analysis

Descriptive statistics were calculated. In order to examine the relationships of the different instruments' (concurrent validity) measures of hydration status as compared to osmolality (OSM), Pearson's product correlations were performed. Thomas et al. defines a perfect correlation as $r = 1.00$, so the values closest to 1.00 will be considered to have the strongest correlation.⁵³ Significance was set at $\alpha \geq .05$. To effectively achieve the necessary power ($1-\beta=0.95$) and effect ($f=0.25$ [medium]) for this investigation, a minimum of 100 samples were needed.

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CHAPTER 4

MANUSCRIPT

Digital and Clinical Refractometers are Valid Instruments for the Measure of Hydration Status

Introduction

Proper levels of hydration are important for normal physiological function of all body systems.^{1,2} Decreased levels of hydration, or hypohydration, create detrimental physiological changes that predispose individuals to heat illnesses including exertional heat stroke.^{1,2,7} The physiological changes induced as a result of hypohydration include: increased heart rate, decreased cardiac output, increased physiological strain, increased perceived strain, decreased muscular power, and decreased cognitive performance.^{1,2,8} The combination of these factors can alter thermoregulation during exercise thereby increasing susceptibility to heat illness.^{1,2,7} Therefore, determining hydration status is important for preventing heat illness and enhancing performance.

Many methods exist for hydration status measurement. These methods include: plasma osmolality, plasma volume shifts, urine specific gravity, urine color, urine conductivity, body mass change, thirst, and doubly labeled water.^{2,7,9,10,13,38,42,46-48,54} Urine osmolality is a common laboratory method of measuring hydration status is considered the urinary “gold standard” due its ability to measure solutes in concentration by freezing point depression.⁹⁻¹² Armstrong et al. described urine osmolality as being more accurate than other urinary indices of hydration

because it is not affected by solutes such as glucose, protein, and urea that may be in the urine sample.⁴² Osmolality works by comparing the freezing point of the specimen to the freezing point of water.^{9,10} Essentially, the more solutes dissolved in the specimen, the lower the freezing temperature of the specimen in comparison to the freezing point of water.^{9,10} Various references for osmolality values of euhydration have been reported. Armstrong et al. stated that a euhydrated value from an initial morning sample should be between 805-867mOsm/kg, whereas Oppliger et al. states values less than or equal to 90mOsm/L represent euhydration.^{10,39}

Urine specific gravity (USG) is defined as the ratio of the densities between urine and water.^{9,10,13} USG is generally considered the most practical and cost effective means of measuring hydration status.⁹ Specific gravity is an easy, non invasive, convenient method of measuring hydration.⁹ The range of measure is from 1.002 μ G to 1.030 μ G.^{9,10,13} Values between 1.010 μ G and 1.020 μ G are considered minimal dehydration, and values above 1.020 μ G are considered severe dehydration.^{9,10,13}

Urine specific gravity is measured with several tools including: clinical refractometer, digital refractometer, urinometer, and reagent strips. Clinical refractometry has been found to have a strong correlation with urine osmolality ($r = .87$), ($r = .87$).^{9,42} Investigation into the relationship between urine osmolality and refractometry by Costa et al. yielded a strong correlation ($r = .81$) as well.⁴³ Refractometry can also be done with a digital refractometer. Unfortunately, no research has been done to validate this technique of refractometry. Investigations into the relationship between the urinometer and osmometer have shown moderate correlation ($r = .60$),⁴⁵ and reagent strips have been shown to have at best, a moderate correlation ($r = .647$, $r = .573$) with urine osmolality.^{9,46}

Based on research on hydration status assessment, the American College of Sports Medicine (ACSM), National Athletic Trainer's Association (NATA) the National Collegiate Athletic Association (NCAA) and National Wrestling Coaches Association (NWCA) have given recommendations for hydration testing.^{1, 2, 14} These organizations provide support for many different tools for assessing hydration status.^{1, 2, 14} The variety in recommendations for assessing hydration status may be confusing and troublesome for clinicians. The inconsistencies in recommendations for assessing hydration status can lead to improper assessments of hydration status. Therefore, the purpose of this study is to determine the validity of urine specific gravity via clinical refractometer, digital refractometer, and urinometer as compared to urine osmolality.

Methods

This study was a descriptive diagnostic validity test design. The criterion measure was urine osmolality as measured by an osmometer. The dependent measure was urine specific gravity measured by the urinometer, clinical refractometer, and digital refractometer. Concurrent validity (also known as criterion validity) of each method of USG measurement as compared to the gold standard urine osmolality was assessed.

Participants

We collected 127 samples, from both males and females, (22 ± 4.7 years) from Indiana State University's campus. Exclusion criteria were diabetes, kidney disease, and chronic urinary tract infection for this study. The Indiana State University Institutional Review Board approved the study and participants provided written informed consent to participate in the study.

*Measurements and Instrumentation***Urine Specific Gravity**

An Atago digital hand-held pen refractometer with a range of 1.000 – 1.060 was used to measure urine specific gravity. Calibration was performed by placing the tip of the instrument into distilled water prior to each data collection session. During data collection the tip of the pen refractometer was placed directly into the urine sample cup and the urine specific gravity measure was recorded from the digital display.

A handheld clinical refractometer (Model A300CL; ATAGO Inc., Bellevue, WA) with a range of 1.000 – 1.060 was calibrated with distilled water. In order to obtain measurements, we used a transfer pipette to place a small amount of urine sample on the clear daylight plate of the refractometer and urine specific gravity measures were recorded to the nearest thousandth.

A Fischer brand urinometer, with a USG measurement range of 1.000-1.040 in.001 increments was utilized. A room temperature (20°C-22.2°C) urine sample (20ml) was then poured from a clean urine sample container into a labeled graduated cylinder.⁴⁴ As the ballast was released into the sample, it was gently spun and not allowed to touch the sides of the graduated cylinder.⁴⁴ The shot ballast sank, displacing the urine around it and the specific gravity was recorded from the area where a meniscus formed around the stem of the ballast.⁴⁴

Osmolality

Osmolality was measured via osmometer (Advanced Micro – Osmometer Model 3320; Advanced Instruments Inc, Norwood, MA). The osmometer was calibrated before each data collection session, as needed, and according to manufacturer's instructions using known calibration standards. Osmometer range was 0-2000 mOsm/kg H₂O. In order to obtain

osmolality measures, approximately 20 μ L of bubble free sample was extracted via osmolality sampler. Once the sample was collected the sampler was cleaned free of any clinging droplets and then placed into the sample port within the operating cradle. We performed measurements of osmolality in duplicate. If sample values were greater than five mOsm/kg H₂O apart, the analysis was performed in triplicate and averaged.

Procedures

Participants provided informed consent and completed a health questionnaire (self report height, weight, gender, void of the day, and presence of any of the following: diabetes, chronic urinary tract infection, menstruation, kidney disease, or the use of supplements or vitamins). Upon completing the health questionnaire, participants were given a clean urine specimen cup and asked to proceed to the restroom to provide as much urine as possible. We assessed hydration status within two hours of sample collection. In order to reduce the risk of contamination, new osmolality tips and transfer pipettes were used for each hydration assessment. Additionally, proper sanitization of each instrument occurred after each measurement. At the end of data collection urine samples were properly disposed

Statistical Analysis

Descriptive statistics were calculated for each participant. In order to examine the relationships of the different instruments' (concurrent validity) measures of hydration status as compared to osmolality, Pearson's product correlations were performed. Thomas et al. defines a perfect correlation as $r = 1.00$, so the values closest to 1.00 will be considered to have the strongest correlation.⁵³ Significance was set at $\alpha \leq .05$. To effectively achieve the necessary

power ($1-\beta=0.95$) and effect ($f=0.25$ [medium]) for this investigation, a minimum of 100 samples were needed.

Results

Strong significant correlations were identified for the digital refractometer ($r=0.814$, $p<0.001$) (Figure 2) and handheld clinical refractometer ($r=0.943$, $p<0.001$) with osmolality (OSM) (Figure 3). A weak statistically insignificant correlation was established between the midjet urinometer ($r=0.133$, $p<0.142$) and OSM (Figure 4). Average hydration status indicated variability among some of the instruments: digital refractometer $USG=1.0194\pm0.0075$, clinical refractometer $USG=1.020\pm0.007$, urinometer $USG=1.028\pm0.091$, osmometer $OSM=743\pm271$)

Discussion

Digital Refractometry

Many investigators have investigated the validity of measuring hydration status with clinician friendly tools. However, to the authors' knowledge, there have been no investigations into the validity of digital refractometry for assessing hydration status. The findings of this study provide positive evidence advocating the use of digital refractometry by practicing clinicians as it showed a strong positive correlation ($r=0.814$, $p<0.001$) with the gold standard osmometer. Our strong "r" value was most likely due to strong methodological choices such as calibration every 10-15 samples as well as prior to each data collection session. Additionally, there are no possibilities for human error when taking readings from the digital display, only simple data recording. Lastly, the prism should refract light the same each test, providing consistent results. Digital refractometers are fast, easy to use, tools that require little more than distilled water for

calibration. Due to their ease of use clinicians who perform frequent hydration status assessments should consider utilizing digital refractometers in their practice.

Clinical Refractometry

Clinical refractometry is a common method of obtaining urine specific gravity measures. Clinical refractometry is another easy, clinician friendly method of assessing hydration status. Hydration assessment via clinical refractometry allows the clinician to view fluid under normal light, detecting the amount of particles in solution (urine specific gravity) in the fluid.⁹ Clinical refractometry has been found to have a strong correlation with urine osmolality ($r = .87$)⁶ ($r = .97$),⁹ ($r = .81$),⁴³ by previous investigators. The positive results of this investigation are similar to previous investigations as clinical refractometry showed a strong positive correlation with osmometry ($r = 0.943$, $p < 0.001$). As with the digital refractometer, we attribute our strong correlation to frequent calibration prior to and during data collection as well as prism refraction. The preceding findings should then contribute to the body of knowledge available to clinicians seeking to support the use of clinical refractometry. Additionally, our results are applicable to more than just athletes as we had a large sample size from athletes as well as the general population.

Urinometry

The theory of urinometry arises from Archimedes' principle of fluid density and displacement. Essentially, the lower the density of a fluid, the deeper an object will sink in the fluid. By utilizing Archimedes' principle, urinometry provides urine specific gravity measurements during hydration status assessment. The findings in this study are converse to previous investigations into the relationship between the urinometry and osmometry as they

showed a moderate correlation($r = .60$).⁴⁵ Utilizing a urinometer can be cumbersome for practitioners, as it requires thorough cleaning of the graduated cylinder between each assessment, increasing the risk of sample contamination. Additionally, the increments of measure utilized on the shot ballast stem cause readings to be difficult to identify. Due to these imprecise increments of measure, the readings from the urinometer have a greater variability, which was identified with our statistical analysis. We hypothesize that these shortcomings produced the difference in correlations, highlighting the inconsistencies that arise from using the urinometer.

Conclusions

The findings of this investigation have provided evidence that measures of hydration status from both digital and clinical refractometers are strongly correlated with the urinary gold standard of freezing point osmometry. Analysis with a urinometer should not be performed, as it showed a weak correlation with the gold standard osmometer, indicating it might not provide accurate results when used to determine hydration status. Knowing this, clinicians can utilize these tools effectively and confidently in their practice of hydration status assessment.

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Figure 1. Data Collection Procedures

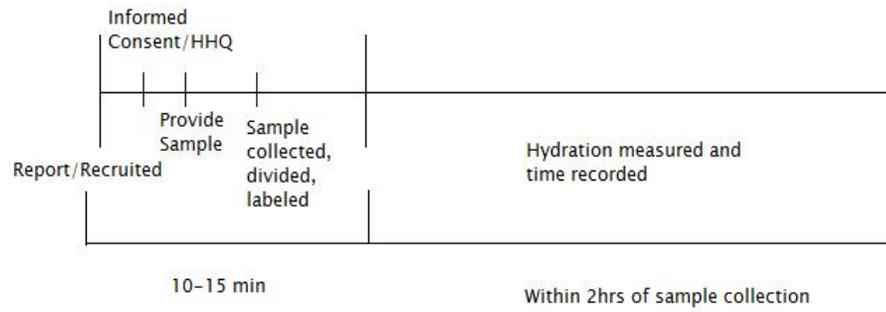
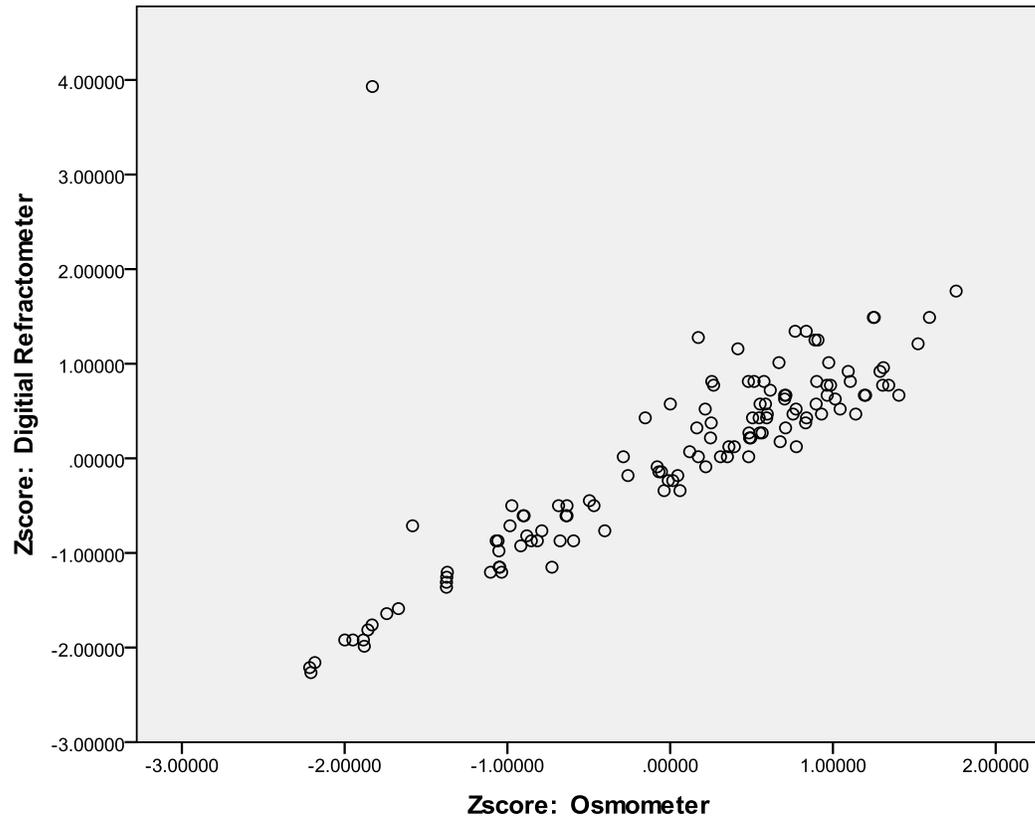


Figure 2. Digital Refractometer and Osmometer



* $r = 0.814$ $p < 0.001$

Figure 3. Clinical Refractometer and Osmometer

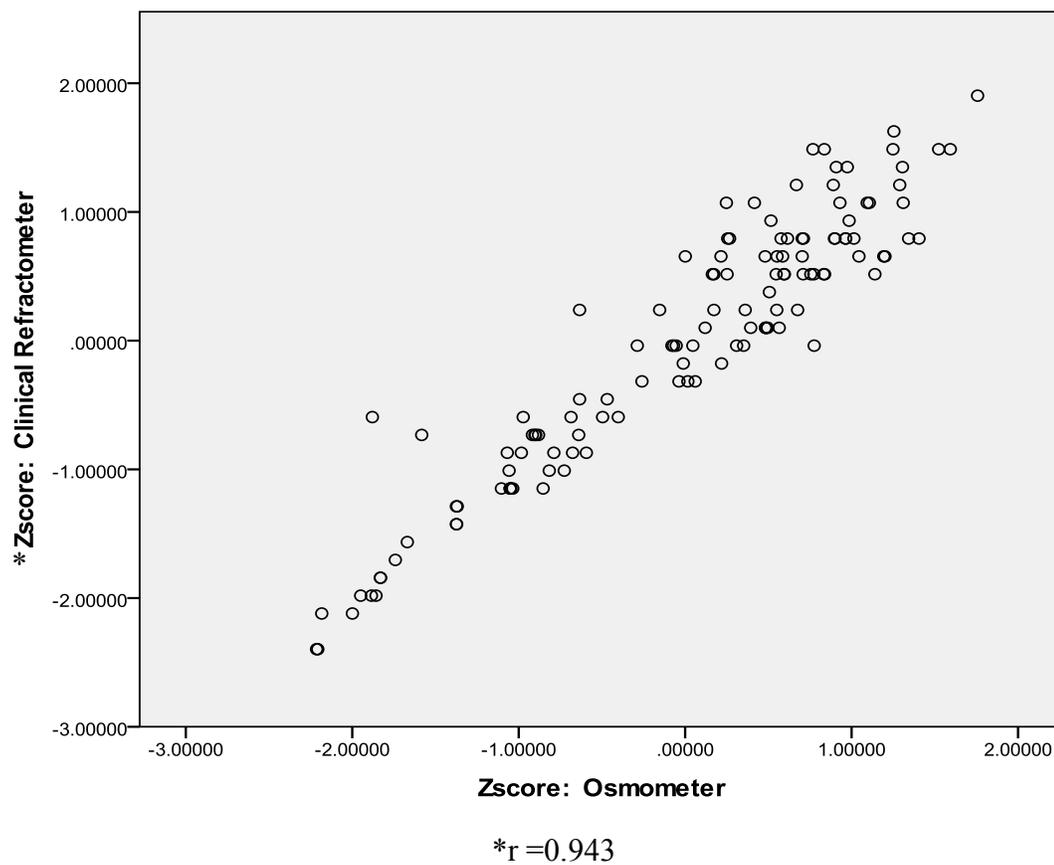
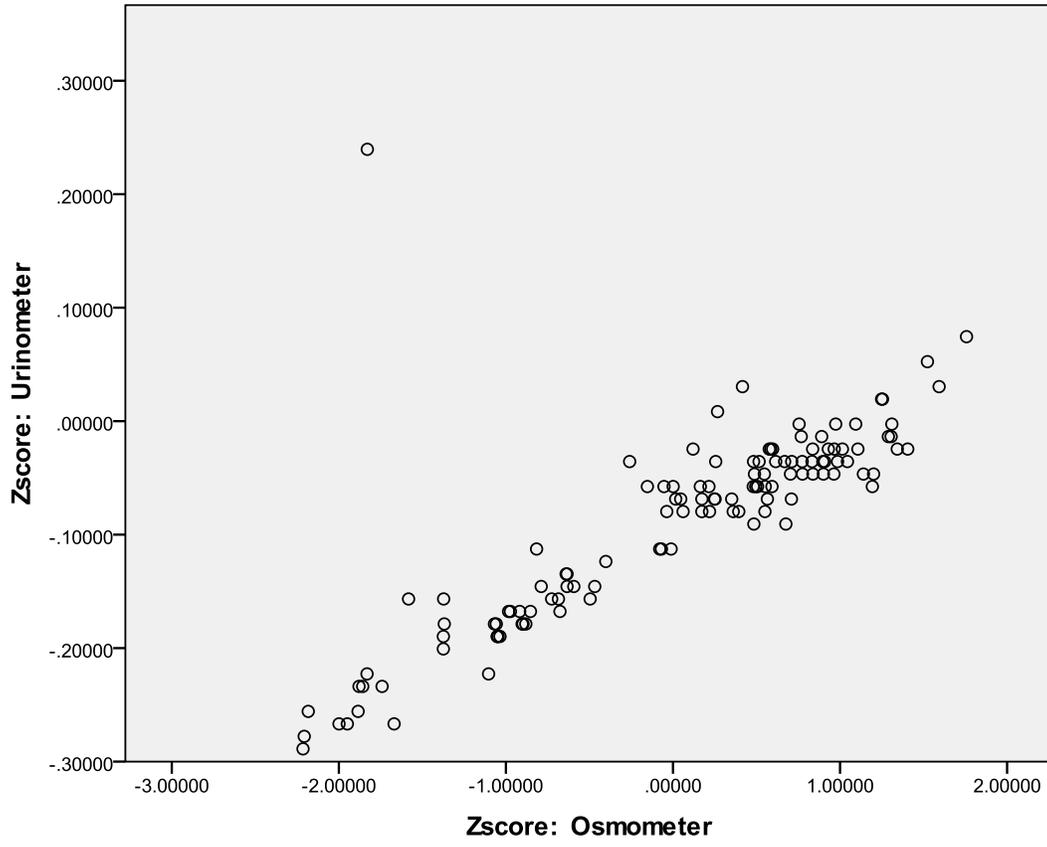


Figure 4. Urinometer and Osmometer



$r = 0.133$ $p < 0.142$

APPENDIX A: STUDY PARAMETERS

Operational Definitions

Clinical Refractometer: A clinical refractometer is a handheld tool for measuring hydration status via urine specific gravity. The practitioner views a urine sample and the concentration of particles are identified on a scale of 1.001 – 1.045. Professional organizations such as ACSM, NATA, NWCA have recommended this type of hydration assessment.

Digital Refractometer: A digital refractometer is a tool for measuring hydration status via urine specific gravity. The practitioner places the tip of the pen style refractometer directly into the sample.

Dehydration: Dehydration is the process of becoming hypohydrated.

Urinometer: A urinometer is a tool consisting of a graduated cylinder and shot ballast used for measuring hydration status via urine specific gravity. This tool works based on Archimedes principle of density and displacement.

Hypohydration: Hypohydration is a state of altered body water below normal limits

Euhydration: Euhydration is a state of total body water balance.

Urine Sample: A urine sample is an amount of urine collected midstream into a sterile container

Urine Specific Gravity (USG): USG is the ratio of the densities between urine and water based on the concentration of particles in solution.

Assumptions

1. Participants will be honest when completing the health questionnaire.

2. There will be variability in hydration status among participants.
3. All levels of hydration will fall within the measureable range of equipment.
4. Participants will understand and follow directions when providing a urine sample.

Delimitations

1. Results are only generalizable to the four specific instruments.
2. Results are generalizable to 18-60 year olds.
3. We will only have knowledge of diseases/conditions that were disclosed or included in the health questionnaire report.

Limitations

1. Specific info on supplements and/or vitamins that are being consumed may not be known.

APPENDIX B: RELEVANT FORMS

WE NEED YOUR HELP!



Purpose: Study the effect of 5 different measurement methods on urine accuracy

Study: You will fill out a confidential questionnaire and provide a urine sample. The total time commitment is approximately **10** mins.

Criteria: Anyone between the ages of 18 and 60 years old is allowed to participate.

Lottery for Prize: You will be entered into a drawing for a chance to win one of 10 **\$20.00** Wal-Mart gift card for participation in the study.

Contact:

Dr. Susan Yeargin
Heather M Adams
Andrew J Niemann

susan.yeargin@indstate.edu	812-237-3962
hadams10@indstate.edu	608-577-1314
aniemann@indstate.edu	515-320-2145



Email Example:

Subject: Hydration Research Project-We need your help!

Dear _____,

This email is in regard to a research project being conducted by Dr. Susan Yeargin, Heather Mata, Dr. Lindsey Eberman, Heather Adams and, Andrew Niemann of Indiana State University.

We are looking for individuals throughout the Terre Haute, Indiana area to volunteer to participate. Involvement in this study is voluntary.

Purpose: Study the effect of 5 different measurement methods on urine accuracy

Study: You will fill out a confidential questionnaire and provide a urine sample. The total time commitment is approximately 10 mins.

Criteria: Anyone between the ages of 18 and 60 years old is allowed to participate.

Lottery for Prize: You will be entered into a drawing for a chance to win one of 10 **\$20.00** Wal-Mart gift card for participation in the study.

Contact: If you have any questions about the study, you may contact Dr. Susan Yeargin at (812) 237-3962 or at susan.yeargin@indstate.edu, Heather Adams at (608) 577-1314 or hadams10@indstate.edu, or Andrew Niemann at (515) 320-2145 or at aniemann@indstate.edu, or. If you have any questions about your rights as a research participant, you may contact the Indiana State University Institutional Review Board (IRB) by mail at Indiana State University, Office of Sponsored Programs, Terre Haute, IN 47809, by phone at (812) 237-8217, or by e-mail at irb@indstate.edu.

Thank you,

Dr. Susan Yeargin
Dr. Lindsey Eberman
Heather Mata
Heather M Adams
Andrew J Niemann



Subject #

Health Questionnaire

Study Title: The Effect of Instrument Type on the Measure of Hydration Status

Height: _____ in Mass: _____ lbs Age: _____ yrs Gender: M or F

Questions		
1. Is this your first time urinating today? If not please list how many times you have urinated today _____	Yes	No
2. Have you been diagnosed with diabetes?	Yes	No
2. Do you have a history of chronic urinary tract infections?	Yes	No
3. Have been diagnosed with kidney disease?	Yes	No
4. Are currently taking any supplements or vitamins?	Yes	No
5. Approximately how much have you exercised in the past 24 hours?	_____ hours	
6. <i>Females only</i> - Are you currently menstruating?	Yes	No

Please answer the questions to the best of your knowledge:

* This information is confidential and will be used for descriptive purposes only. This information will not exclude you from the study or lottery.



CONSENT TO PARTICIPATE IN RESEARCH

Validation of Urine Hydration Status Measurement Methodology: A Five Part Investigation

You are asked to participate in a research study conducted by Dr. Susan Yeargin, Dr. Lindsey Eberman, Heather Mata, Heather Adams, and Andrew Niemann, members of the Department of Applied Medicine and Rehabilitation at Indiana State University. Your participation in this study is voluntary, so at any time, you can discontinue without any consequences. Please read the information below and ask questions about anything you do not understand, before deciding whether or not to participate.

- **PURPOSE OF THE STUDY**

Urine is commonly used to determine a person's hydration status by researchers and health care providers. Current research is unclear about the best ways to evaluate a urine sample. The goal of this study is to determine whether factors like time, shaking, temperature, number of times urinating, and measurement type change the results of a urine sample.

- **PROCEDURES**

If you volunteer to participate in this study, you will be asked to do the following things:

- Complete a health questionnaire
- You will be given a clean urine specimen cup
- Go to the restroom with the cup, making sure to lock the door behind you
- Provide as much urine as possible in the sample cup
- Wash your hands and leave the urine sample in the restroom for the researchers to analyze later

- **POTENTIAL RISKS AND DISCOMFORTS**

We expect the risks for this study will be minor. If your discomforts become a problem, you may choose to discontinue your participation at any time. Possible risks that may be experienced include you becoming socially uncomfortable due to the process of urine collection and transportation of urine. Allowing you to leave your sample in the bathroom will help minimize this risk.

- **POTENTIAL BENEFITS TO SUBJECTS AND/OR TO SOCIETY**

It is unlikely you will directly benefit from participation in this study. However, this research will help increase the awareness and education on the importance of hydration in addition to generating standardized procedures, for both clinical and research purposes, for assessing hydration status.

- **PAYMENT FOR PARTICIPATION**

If you choose to participate, you can also choose to enter a lottery for a \$20 Wal-Mart gift card. Ten gift cards will be distributed at the conclusion of the study based on a random drawing of email addresses. Please indicate below whether you would like to be included in the lottery. If you choose not to enter the lottery, you can still provide a urine sample for analysis. You can also choose to provide more than one sample, but your name will only be entered into the drawing once.

Please note: Foreign nationals on visas other than F-1 or J-1 may not be eligible to receive payment for participation in this study.

Place a check in the box to indicate your choice:

I DO want to enter my name in the lottery.
enter into the lottery.

I DO NOT want to

Email address: _____

- **CONFIDENTIALITY**

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law. Confidentiality will be maintained by means of assigning you a subject number. The only location where your subject number and name will be together will be in a file on the primary investigator's password protected computer. Only the investigators will have access to this file. This consent form (which only has your name) and the health questionnaire (which only has your subject number) will be stored in a locked cabinet in a locked office in the Applied Medicine Research Laboratory. Only the primary investigators will have access to these files. If you choose to discontinue participation at any time, all forms related to your participation will be immediately destroyed.

- **PARTICIPATION AND WITHDRAWAL**

You can choose whether or not to be in this study. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind or loss of benefits to which you are otherwise entitled. You may also refuse to answer any questions you do not want to answer. There is no penalty if you withdraw from the study and you will not lose any benefits to which you are otherwise entitled.

• **IDENTIFICATION OF INVESTIGATORS**

If you have any questions or concerns about this research, please contact

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• **RIGHTS OF RESEARCH SUBJECTS**

If you have any questions about your rights as a research subject, you may contact the Indiana State University Institutional Review Board (IRB) by mail at Indiana State University, Office of Sponsored Programs, Terre Haute, IN 47809, by phone at (812) 237-8217, or e-mail the IRB at irb@indstate.edu. You will be given the opportunity to discuss any questions about your rights as a research subject with a member of the IRB. The IRB is an independent committee composed of members of the University community, as well as lay members of the community not connected with ISU. The IRB has reviewed and approved this study.

I understand the procedures described above. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

Printed Name of Subject

Signature of Subject

Date

*Leave this amount of space
for IRB approval stamp (unless
you plan to include the approval
information in the text of the ICD)*

APPENDIX C: RAW DATA

Subject #	Height (in)	Height (centimeters)	Weight (lbs)	Weight (kg)	Age	Gender
1	75	190.5	215	97.72727 273	23	Male
2	73	185.42	200	90.90909 091	30	Male
3	64	162.56	145	65.90909 091	23	Female
4	62	157.48	140	63.63636 364	22	Female
6	64	162.56	220	100	20	Female
7	62	157.48	120	54.54545 455	22	Female
9	67	170.18	145	65.90909 091	31	Female
12	72	182.88	174	79.09090 909	32	Male
13	69	175.26	180	81.81818 182	28	Male
14	72	182.88	145	65.90909 091	22	Male
15	77	195.58	210	95.45454 545	20	Male
16	73	185.42	160	72.72727 273	18	Male
17	61	154.94	118	53.63636 364	18	Female
18	72	182.88	174	79.09090 909	32	Male
20	67	170.18	130	59.09090 909	18	Male
21	66	167.64	125	56.81818 182	18	Female
22	67	170.18	125	56.81818	19	Male
23	72	182.88	145	65.90909	19	Male

Subject #	Height (in)	Height (centimeters)	Weight (lbs)	Weight (kg)	Age	Gender
24	71	180.34	158	71.8181	19	Male
25	72	182.88	160	72.72727 273	19	Male
26	67	170.18	151	68.63636 364	18	Female
27	70.5	179.07	121	55	19	Male
28	69	175.26	135	61.36363 636	20	Female
29	65	165.1	135	61.36363 636	19	Female
30	66	167.64	133	60.45454 545	20	Female
31	66	167.64	125	56.81818 182	18	Female
32	61	154.94	90	40.90909 091	19	Female
33	74	187.96	215	97.72727 273	23	Male
35	66	167.64	128	58.18181 818	18	Male
36	64	162.56	132	60	21	Female
37	65	165.1	137	62.27272 727	21	Female
38	61.5	156.21	110	50	21	Female
39	70	177.8	135	61.36363 636	21	Male
40	65	165.1	141	64.09090 909	22	Female
41	66	167.64	135	61.36363 636	20	Female
42	72	182.88	150	68.18181 818	19	Male
43	66.5	168.91	116	52.72727 273	20	Female
44	71	180.34	159	72.27272 727	20	Male
45	72.5	184.15	159	72.27272 727	22	Male
46		0	230	104.5454 545	20	Male
47	73	185.42	210	95.45454 545	20	Male

Subject #	Height (in)	Height (centimeters)	Weight (lbs)	Weight (kg)	Age	Gender
48	73	185.42	155	70.45454 545	18	Male
49	71	180.34	170	77.27272 727	18	Female
50	74	187.96	154	70	18	Male
51	71	180.34	185	84.09090 909	19	Male
52	73	185.42	210	95.45454 545	23	Male
53	73	185.42	167	75.90909 091	19	Male
54	72	182.88	172	78.18181 818	32	Male
56	75	190.5	205	93.18181 818	22	Male
57	75	190.5	173	78.63636 364	19	Male
58	69	175.26	162	73.63636 364	18	Male
59	68	172.72	192	87.27272 727	23	Male
60	64	162.56	128	58.18181 818	17	Female
61	67	170.18	134	60.90909 091	19	Female
62	59	149.86	133	60.45454 545	18	Female
63	69.5	176.53	175	79.54545 455	18	Male
64	74	187.96	205	93.18181 818	20	Male
65	72	182.88	220	100	21	Male
66	62	157.48	120	54.54545 455	22	Female
67	70	177.8	160	72.72727 273	20	Male
68	61	154.94	200	90.90909 091	19	Female
69	67	170.18	135	61.3636	23	Male

Subject #	Height (in)	Height (centimeters)	Weight (lbs)	Weight (kg)	Age	Gender
70	72	182.88	200	90.90909 091	21	Male
71	70	177.8	150	68.18181 818	19	Female
72	70.5	179.07	183	83.18181 818	20	Female
73	72	182.88	137	62.27272 727	19	Female
74	70	177.8	136	61.81818 182	18	Female
75	73	185.42	230	104.5454 545	23	Male
76	72	182.88	179	81.36363 636	22	Male
77	86	218.44	187.6	85.27272 727	19	Male
78	67	170.18	125	56.81818 182	20	Female
79	72	182.88	182	82.72727 273	20	Male
80	62	157.48	122	55.45454 545	22	Female
81	63	160.02	123	55.90909 091	21	Female
82	67	170.18	133	60.45454 545	20	Female
83	69	175.26	260	118.1818 182	21	Male
84	66	167.64	135	61.36363 636	20	Male
85	66	167.64	124	56.36363 636	21	Female
86	74	187.96	220	100	23	Male
88	76	193.04	295	134.0909 091	20	Male
89	69	175.26	190	86.36363 636	23	Male
90	68	172.72	134	60.90909 091	19	Female
91	73	185.42	185	84.09	21	Male
92	63	160.02	127	57.72727	19	Female

Subject #	Height (in)	Height (centimeters)	Weight (lbs)	Weight (kg)	Age	Gender
93	67	170.18	145	65.90909091	23	Male
95	68	172.72	135	61.36363636	19	Female
96	69	175.26	190	86.36363636	23	Male
99	65	165.1	170	77.27272727	27	Female
100	70	177.8	190	86.36363636	29	Male
102	67	170.18	245	111.36363636	26	Male
104	72	182.88	174	79.09090909	32	Male
106	67	170.18	150	68.18181818	34	Female
108	74	187.96	220	100	23	Male
110	66	167.64	215	97.72727273	21	Female
111	69	175.26	210	95.45454545	19	Male
112	64	162.56	140	63.63636364	19	Female
113	69	175.26	130	59.09090909	21	Male
114	72	182.88	165	75	20	Male
116	73	185.42	215	97.72727273	23	Male
117	74	187.96	220	100	23	Male
120	66	167.64	245	111.36363636	26	Male
121	68	172.72	210	95.45454545	23	Female
122	68	172.72	155	70.45454545	21	Female
123	71	180.34	178	80.90909091	22	Male
124	64	162.56	130	59.09090909	18	Female
125	70	177.8	200	90.90909091	21	Male
126	62	157.48	123	55.90909091	22	Female

Subject #	Height (in)	Height (centimeters)	Weight (lbs)	Weight (kg)	Age	Gender
127	75	190.5	190	86.36363 636	37	Male
	Mean	173.5431 481		75.30976 431	21.50925 926	63
	SD	20.15338 22		17.42828 95	3.848765 721	45

Raw Data

Sample #	<2hrs(control)					
	DR	CR	Ur	Osmo		
1	1.0288	1.03	1.025	988	911	
2	1.049	1.007	1.05	247	246	
3	1.0252	1.026	1.026	1106	1109	
4	1.0148	1.015	1.016	569	570	
6	1.0226	1.023	1.023	878	883	
7	1.0128	1.013	1.012	455	457	
8	1.0288	1.029	1.027	990	983	981
9	1.016	1.016	1.014	607	610	
10	1.0156	1.017	1.015	615	617	
11	1.0061	1.007	1.008	247	245	
12	1.0195	1.02		662	668	665
13	1.0049	1.006	1.005	232	231	
14	1.0241	1.026	1.026	1025	1013	1017
15	1.0176	1.018	1.022	748	747	
16	1.0226	1.024	1.024	895	888	
17	1.021	1.028	1.022	816	807	808
18	1.0195	1.02	1.022	841	836	
19	1.0203	1.021	1.021	861	842	847
20	1.0128	1.013	1.018	521	522	
21	1.0281	1.028	1.031	857	855	
22	1.0327	1.034	1.035	1238	1206	1216
23	1.0226	1.024	1.024	973	968	
24	1.0244	1.026	1.024	939	930	933
25	1.0049	1.005	1.004	200	201	
26	1.0091	1.01	1.011	370	370	
27	1.0295	1.031	1.026	972	968	
28	1.0222	1.024	1.022	811	812	
29	1.0136	1.014	1.015	531	527	
30	1.0057	1.006	1.007	239	239	
31	1.014	1.015	1.014	312	315	
32	1.021	1.021	1.023	877	878	
33	1.0023	1.003	1.003	144	144	
34	1.0027	1.003	1.002	141	143	
35	1.0233	1.025	1.025	1025	1028	
36	1.0107	1.013	1.014	550	543	545
37	1.0168	1.018	1.021	728	736	735
38	1.0148	1.022	1.016	570	572	
39	1.0263	1.029	1.027	1096	1088	1095
40	1.0136	1.016	1.017	634	634	

Sample #	<2hrs(control)					
	DR	CR	Ur	Osmo		
	1.0044	1.016	1.007	233	233	
41	1.0195	1.021	1.025	874	874	
42	1.0237	1.025	1.023	895	891	
43	1.0252	1.027	1.025	1012	1009	
44	1.0128	1.014	1.015	580	584	
45	1.0295	1.031	1.027	950	953	
46	1.0244	1.026	1.026	1004	1006	
47	1.0237	1.025	1.023	737	746	747
48	1.0168	1.018	1.021	758	761	
49	1.0237	1.025	1.026	900	904	
50	1.0255	1.027	1.025	882	884	
51	1.0263	1.028	1.028	1038	1042	
52	1.0218	1.024	1.022	933	938	
53	1.0183	1.02	1.023	730	727	
54	1.0183	1.02	1.018	721	727	725
55	1.027	1.029	1.025	926	923	
56	1.027	1.03	1.028	1009	1006	
57	1.0266	1.028	1.028	1103	1095	1098
58	1.0244	1.026	1.025	932	939	937
59	1.0233	1.025	1.023	799	804	
60	1.0195	1.022	1.022	790	790	
61	1.0237	1.026	1.025	984	989	
62	1.0285	1.031	1.033	1157	1156	
63	1.0255	1.028	1.026	1041	1046	
64	1.0226	1.022	1.023	703	700	
65	1.0222	1.024	1.025	975	964	968
66	1.0132	1.015	1.012	506	502	
67	1.0244	1.026	1.026	1123	1126	
68	1.018	1.02	1.022	752	758	757
69	1.0031	1.005	1.005	150	151	
70	1.0229	1.028	1.026	997	994	
71	1.0203	1.022	1.021	839	843	
72	1.0241	1.025	2.024	936	932	
73	1.0218	1.024	1.023	787	788	
74	1.0252	1.03	1.027	1096	1099	
75	1.018	1.018	1.025	668	677	673
76	1.029	1.024	1.021	790	790	
77	1.0187	1.02	1.018	721	722	
78	1.0255	1.026	1.026	899	900	
79	1.0229	1.024	1.026	904	912	899
80	1.0156	1.017	1.015	573	569	

Sample #	<2hrs(control)					
	DR	CR	Ur	Osmo		
81	1.0156	1.016	1.013	480	478	
82	1.0306	1.031	1.031	1176	1175	
83	1.014	1.014	1.013	476	476	
84	1.0128	1.012	1.013	511	512	
85	1.0229	1.024	1.028	947	949	
86	1.0233	1.024	1.025	951	955	
87	1.0195	1.02	N/A	828	826	
88	1.0128	1.014	1.013	558	561	
89	1.0128	1.014	1.012	452	454	
90	1.0156	1.016	1.014	556	558	
91	1.0187	1.019	1.021	800	805	
92	1.0095	1.011	1.01	369	371	
93	1.0099	1.01	1.014	370	371	
94	1.012	1.012	1.011	459	456	
95	1.0148	1.015	1.012	500	499	
96	1.0148	1.015	1.012	500	496	
97	1.0124	1.015	1.013	494	494	
98	1.0049	1.006	1.004	213	215	
99	1.0252	1.026	1.029	816	815	
100	1.0255	1.026	1.025	813	812	
101	1.0244	1.025	1.024	1069	1069	
102	1.0244	1.025	1.023	1067	1067	
103	1.021	1.021	1.024	874	877	
104	1.0214	1.021	1.02	875	874	
105	1.0103	1.011	1.012	371	372	
106	1.0214	1.022	1.021	894	891	
107	1.0214	1.021	1.022	896	897	
108	1.0255	1.026	1.024	990	985	
109	1.0107	1.012	1.011	459	459	
110	1.0203	1.02	1.024	951	956	
111	1.0248	1.026	1.025	916	907	907
112	1.0306	1.032	1.03	1086	1081	
113	1.0199	1.021	1.026	771	778	778
114	1.0306	1.031	1.03	1087	1079	1080
115	1.0207	1.022	1.02	926	927	
116	1.0252	1.026	1.024	1006	1003	
117	1.0103	1.012	1.011	464	460	
118	1.0107	1.012	1.011	459	457	
119	1.0229	1.024	1.024	1050	1055	
120	1.0176	1.019	1.018	741	739	
121	1.007	1.008	1.007	271	270	
122	1.0255	1.025	1.023	874	873	

APPENDIX D: STATISTICAL ANALYSIS

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Digital Refractometer	125	1.0023	1.0490	1.019374	.0075373
Clinical Refractometer	125	1.003	1.034	1.02028	.007208
Urinometer	123	1.002	2.024	1.02824	.090854
Osmometer	125	142	1220	743.09	271.423
Valid N (listwise)	123				

Correlations

		Zscore: Digital Refractometer	Zscore: Osmometer
Zscore: Digital Refractometer	Pearson Correlation	1	.814**
	Sig. (2-tailed)		.000
	N	125	125
Zscore: Osmometer	Pearson Correlation	.814**	1
	Sig. (2-tailed)	.000	
	N	125	125

** . Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Zscore: Osmometer	Zscore: Clinical Refractometer
Zscore: Osmometer	Pearson Correlation	1	.943**
	Sig. (2-tailed)		.000
	N	125	125
Zscore: Clinical Refractometer	Pearson Correlation	.943**	1
	Sig. (2-tailed)	.000	
	N	125	125

** . Correlation is significant at the 0.01 level (2-tailed).

APPENDIX E: RECOMMENDATIONS

Recommendations for methodological improvement:

- Establish inter-rater reliability for clinical refractometer due to measures being assessed by more than one investigator.
- Establish intra-rater reliability for the urinometer
- Assess the temperature of each sample to see if they were all of similar temperature at the time of assessment

Recommendations for further research:

- Investigate the relationship between urine color and digital and clinical refractometers. This may provide more insight into the practicality of urine color assessment by athletes.
- Investigate clinical refractometry sensitivity to acute hydration post practice
- Attempt to measure the amount of athletes who show up for preseason weigh ins with euhydrated baseline body masses by assessing hydration status with digital and clinical refractometers as well as an osmometer.