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Determining Whether a Spot Urine Sample is an Accurate Indication of a Person's Hydration Status

Hannah Glidewell

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Hannah Glidewell

The University of Arkansas

Main Advisor: Brendon McDermott, Ph.D.

Committee Members: Matthew Ganio, Ph.D. and Lesley Vandermark, Ph.D.

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Chapter I: Introduction

Optimal hydration is necessary for each individual and is specific depending on their lifestyle choices. Whether the goal is to maintain athletic performance or to fuel the body for proper physiological function, hydration is a key component. If hydration levels drastically deviate from normal, it could be detrimental to a person's health (McDermott et al., 2017). Heat loss through perspiration is the bodies main way to expel heat (McDermott et al., 2017). Internal body temperature will rise with exercise secondary to metabolic heat production. This effect is exacerbated if a person is dehydrated and during activity in a warm environment. This could increase stress on cardiovascular function and be a contributing factor for heat stroke (McDermott et al., 2017). Contrarily, hyponatremia may occur, which is having an excess amount of fluid in the body, leading to swelling in cells and organs if an individual intakes too much fluid. Some conditions are potentially fatal depending on the severity (McDermott et al., 2017). Therefore, keeping hydration levels within a normal range is important to reduce the risk of several complications and to aid in maintaining good health. This supports the need to have accurate measurement techniques to assess an individual's hydration status either in a controlled laboratory or in everyday living conditions (Armstrong, Lawrence 2007).

There exists debate over the best way to measure hydration. The "gold standard" for hydration assessment is assumed to be the measurement of total body water (Armstrong, Lawrence 2007). Experts claim this is the most accurate technique in a laboratory setting due to the body being in a state of equilibrium and the control of confounding variables (Armstrong, Lawrence 2007). There are other methods that have been tested to detect hydration levels, such as first-morning urine samples, 24-hour urine samples, and spot urine samples. First-morning samples can provide a good estimate since they are taken at a state of equilibrium, but they tend

to be over concentrated (Bottin et al., 2016). Spot urine samples have shown to be a good measurement in testing hydration status at a specific time point, but it is speculated that they are easily influenced by fluctuation in water intake or exercise during the day (McDermott et al., 2017). A urine sample over a 24-hour period is an accurate method since it includes all intake and output, yet it is time consuming and cumbersome for an individual to conduct (Bottin et al., 2016). In addition to laboratory techniques, there are many variables that make it difficult to conclude a single "gold standard" for hydration assessment in an everyday environment. Many believe the best detection, in this case, is to measure a change in body weight along with urine color and thirst. This is a simple and quick measurement that any individual can assess on their own (Armstrong, Lawrence 2007). The goal of this study is to determine what technique will be an accurate measurement of a person's hydration status. The research question includes the comparison of validity of a spot urine sample and a first morning sample compared to a 24-hour urine sample for identifying individual hydration status. The proposed hypothesis is that a spot urine sample compared to a first- morning sample will give the most accurate results of the participant's hydration levels in comparison to a 24- hour urine sample assigned as the standard hydration status in this study.

Chapter II: Literature Review

The Importance of Hydration

Water composes 50-60% of adult body weight and is essential for human life and function. As the main component of the human body, water has thermoregulatory, solvent, and carrier properties to aid in many functions of the body. It is crucial to maintain hydration levels through replenishment during the day (Bottin et al., 2016). A person's water intake can be

influenced by body size, level of activity, diet, metabolic rate, the climate they live in, and urine concentration (Perrier et al., 2013).

Adequate water intake is based off an average survey of the population; therefore, it is difficult to determine an individual's hydration needs from mean values due to the potential influencing factors (Perrier et al., 2013). Measurement of urine concentration alone is hard to determine water requirements due to factors such as climate, diet, and activity levels; yet, urine samples can aid in assessing the adequacy of someone's hydration behaviors (Bottin et al., 2016). Required fluid intake will differ among individuals and be dependent on the amount of fluid ample enough to replace the water lost (Cheuvront, Kenefick 2016). Water is constantly lost through excretion by the kidneys, respiration, and perspiration (Perrier et al., 2016). Mild dehydration has been shown to decrease alertness, affect cognitive functioning, and increase heart rate and core body temperature to dangerous levels. (Wilson, Morley 2003). It can also affect the body on a cellular level which could include metabolic function, hormone transport and stimulation, and cell apoptosis (Lang et al., 1998). In contrast, if excess water is consumed, hyponatremia could occur due to over dilution of bodily fluids to decrease the concentration of sodium present in the blood (Noakes 2013). Dehydration and hyponatremia may both have dangerous side effects; therefore, assessing an accurate level of hydration status for a given individual is important for optimal body functioning and the health of the individual.

Hydration Terminology

It is difficult to designate a specific value to dehydration or hypohydration due to inconsistency in water intake and different activity levels of individuals (Armstrong et al., 2010). Euhydration is defined as having normal total body water. In determining whether one is

drinking enough water, it has been concluded that a status of euhydration is possible if the total body water does not drop below >1% and a balance of fluid intake and loss is achieved (Cheuvront, Kenefick 2016).

Dehydration is the process of losing water below the average total body value. Water is lost through urination, sweat, or feces (Armstrong, Lawrence 2007). Hypohydration is defined as a state where total body water levels are lower (Armstrong, Lawrence 2007). Hypohydration can occur due to acute or chronic dehydration (McDermott et al., 2017). Therefore, athletes or occupational workers in the heat often become dehydrated. It has been concluded that loss of body weight, level of thirst, increased plasma osmolality, and urine concentration examination can together be a reliable platform to test hydration status (Cheuvront, Kenefick 2016).

Assessment Techniques

The most accurate way to assess hydration status has been debated and analyzed through many different studies (Armstrong, Lawrence 2007; Bottin et al., 2016; Cheuvront, Kenefick 2016; McDermott et al., 2017). The most common ways to assess hydration can be through a 24 -hour urine sample, first- morning sample, or a spot sample of that given day. In a recent study, it was speculated that a spot urine sample is not an accurate way to assess hydration (Cheuvront, Kenefick 2016). Cheuvront claims that the timing of the urine sample is the most important factor. By testing a sample in its most steady state, which is independent of influential factors such as diet, activity, and water intake, the most accurate results will be concluded (Cheuvront, Kenefick 2016). It is assumed that any influencing variables are resolved overnight while there is no fluctuating water intake and the body's fluids are at equilibrium. Therefore, it was concluded

that a urine sample from the first void of the morning would most accurately depict the individual's hydration status.

Debate of Assessment Techniques

These findings propose the best way to measure a subject's hydration status in "real time." Spot samples to test an individual's hydration status at a specific time before or after activity is a common practice. However, a person's true hydration state will differ between a first-morning sample and a spot sample taken later in the day. It has been suggested that a spot sample taken at any time without previous intake being controlled will not detect true hydration status. It is true that when there is fluctuating fluid intake, it will skew the urine concentration values to alter hydration results (Maughan, Shirreffs 2008). Due to this alteration in concentration, the spot urine sample concentration has the potential to show inaccurate findings compared to the first morning sample. Gowans and Fraser claim that their findings indicate there is a significant difference between the spot samples and 24-hour samples and little difference between 24-hour samples and first morning samples (Gowans, Frasier 1987). If the first-morning sample is the most accurate in a steady state, and the spot samples differ from these values, then the spot samples are dependent on water intake and are not accurate for hydration status testing. In contrast to using a spot sample, it is also claimed that a 24-hour sample is not an accurate measure. In this case, a high solute concentration does not indicate dehydration since there has been no indication of a normal 24-hour concentration range for an individual (Kamel et al., 1990).

Cheuvront claims the best way to self-assess a person's hydration status is to use the first morning sample color combined with body mass and thirst levels. Yet, this method does not test

for true hydration status at the onset of exercise or an activity. These measurements also depend on subjective judgement in assessing the color of urine or levels of thirst. He claims that assessing body weight is the most accurate way to test a mid-day status (Cheuvront et al., 2015). Little evidence is provided using the method of spot samples for accurate measurements and have not been convincingly refuted.

Hahn has conducted a study testing the accuracy of spot samples amidst a healthy population (Hahn, Waldréus 2013). A spot sample was taken among 300 workers to examine their urine specific gravity, osmolality, color, and fluid retention. Each of the workers were instructed not to ingest any fluids at least 2 hours prior to giving their spot sample, so a steady state sample could be produced. The focus of the study was to assess the fluid retention index of the participants and how it indicated their hydration status. It was concluded that the time of day the urine sample was obtained did not influence the results (Hahn, Waldréus 2013). The only effect that was differential was whether the subject followed the allotted time of fluid restriction before their sample was given. The 2-hour period of fluid restriction is an important factor because it is believed to have closely resembled a steady state of the bodies hydration state which is necessary for accurate results (Hahn, Waldréus 2013). It was determined that a spot sample could be used as a proper way to assess hydration status and fluid retention in individuals at any given time if a fluid restriction has taken place before the sample to resemble a steady state.

It has been assumed that a spot sample may produce false positive or negative results due to uncontrolled water intake, and that a first-morning urine sample would provide more accurate results due to no fluid intake overnight (Cheuvront et al., 2015). This is easily controlled in a spot sample by restricting fluids before the sample is given for a specific amount of time to imitate the same steady state that is produced overnight in the first-morning urine concentration.

A first- morning sample combined with body mass only provides a "day to day" baseline value for hydration. When a subject's hydration levels fluctuate throughout the day before or after activity, the preferred method for self- assessment has been to measure the individual's body weight to account for the loss of water (Cheuvront et al., 2015). Whereas, a spot sample could also provide indication of a mid-day hydration status based on four urinary biomarkers, which could be more accurate than just measuring body weight. Hahn states that the time of day of the spot sample does not matter if a steady state is produced before testing (Hahn, Waldréus 2013).

Afternoon spot samples have also been compared to 24-hour urinary samples to see if they have consistent findings. Previous research shows that a first- morning urine sample is overly concentrated to be inconsistent with 24 -hour values, which contradicts the idea of using first-morning samples for accuracy. Morning urine samples overestimate a typical 24- hour value due to the body fasting from food or water during sleep (Bottin et al., 2016). Although the sample is overly concentrated, this indicates normal values of a functioning kidney during antidiuresis to allow for water conservation of the body. As stated previously, spot samples have been analyzed and may tend to be a good indication of hydration levels when water intake has been tightly controlled. Yet, little research has shown the comparison between spot samples and 24- hour samples under normal living conditions without control.

Bottin conducted a study to analyze what time of day a 24-hour sample and spot sample would be most equivalent in concentration levels. These researchers predicted that an afternoon sample would be more consistent than a morning or evening sample. During the trial, participants were instructed to maintain normal dietary habits and record their intake. Each individual void throughout the day was recorded and analyzed as well as the pooled urine samples to measure the 24-hour concentration. Results showed that the afternoon, and 24-hour

samples were equivalent, meaning there was <100mOsm/kg difference from the 24-hour value findings. The morning/midday samples were taken in the hours of 1000-1400. The afternoon samples were in the time frame of 1401-2000 hours. The mean difference from 1000-1200 hours was 82; 107 from 1201-1400 hours; -25 from 1401-1600 hours; 29 from 1601-1800 hours; and 12 from 1801-2000 hours (Bottin et al., 2016). The mean difference was taken from the 24-hour sample, and it was concluded that the 1000-1400 hours were not equivalent and 1401-2000 was equivalent (Bottin et al., 2016).

Collection of urine over a 24-hour period can be demanding for free-living conditions, so an afternoon spot sample would be more convenient to test hydration status. Even though first-morning samples are popularly used by healthcare professionals, it has been recorded that first morning samples overestimate a 24-hour concentration to show normal kidney function. In the healthcare setting, a morning void should not be used in place of a 24-hour urine collection due to its elevated concentration. Instead, an afternoon spot sample could be used in patient settings to increase efficiency and accuracy (Bottin et al., 2016). It was concluded that using an afternoon spot sample to test Uosm and Usg is equivalent to 24-hour values and is a more efficient alternative to assess hydration levels in free-living individuals. (Bottin et al., 2016).

It was also concluded by Perrier that spot afternoon spot samples can easily replace 24-hour urine collections. The goal of the study was to observe hydration biomarkers in response to water influx. Values within +/- 50 mOsm/kg of the 24-hour collection findings were considered equivalent. Results showed that early or late afternoon samples taken within the 1600-2000 hours were 87% consistent with 24-hour urine concentrations falling within the +/-50 mOsm/kg range. Morning samples were only 46% consistent and overnight samples were even lower at 37% (Perrier et al., 2013). It was concluded that the morning samples were significantly more

concentrated than the other spot samples (Perrier et al., 2013). Hydration biomarkers were shown to rapidly change in response to water influx, yet the afternoon samples still reflected the 24-hour collections with more accuracy.

Chapter III: Methods

Research Question: Will a spot urine sample or a first- morning urine sample give the most valid results of the participant's hydration levels when compared to a 24- hour urine sample assigned as the standard hydration status in this study?

Research Design: Double blind, counterbalanced, randomized controlled trial

Independent Variables: Exercise during trials, hydration status leading into trials, diet logs (macronutrient intake), and alcohol and caffeine intake.

Dependent Variables: USG and Urine Osmolality at the time points of:

- 1st morning urine sample leading into the trial and the following day
- Spot urine samples the day of the trial, post-trial, 3-hour post-trial, and the following day
- 24-hour urine sample prior to the trial
- 16-hour urine sample post-trial

Comparison of Different Time Points Measured

The 24-hour sample included all urine output prior to the exercise trial, as well as the first-morning spot and the pre-trial spot urine samples. The 16-hour urine sample included all urine output from post exercise, the first-morning, and spot sample.

- Pre-trial 24-hour vs. Pre-trial spot sample
- Pre-trial 24-hour vs. Pre-trial first-morning sample
- Post-trial 16-hour vs. Post-trial spot sample

- Post-trial 16-hour vs. Post-trial first-morning sample

Participants

The participants in this study included 20 healthy, non-smoking males that exercised habitually (at least 3 days per week) (age: 23.50 ± 2.32 years, height: 179.14cm ± 6.09cm, weight: 87.51kg ± 15.27kg, percent body fat 24.78 ± 8.89). They were asked to refrain from alcohol at least 24 hours prior to their trial and refrain from caffeine for at least 12 hours prior.

Measures/Instruments

Body composition and bone density were measured via dual-energy X-ray absorption (DEXA). Diet logs were printed and given to participants along with urine containers for 24-hour urine collection. The duration of work took place in an environmental chamber (30°C and 55% relative humidity). Urine osmolality was assessed via freezing point depression osmometer (Advanced Instruments Inc. Model 3250) . USG was measured via refractometry (ATAGO).

Procedure

Twenty-four hours before the first exercise trial, the participant received a baseline assessment to measure body composition and bone density. They were asked to collect all urine 24- hours before their trial with the first- morning urine sample separate in a small collection cup. The 24- hour urine sample, first- morning urine sample, and diet log were given to the researcher the next day. The participant was also asked to fill out a 24- hour history questionnaire to confirm compliance regarding sleep and the lack of alcohol and caffeine consumption,

The next day, the participant was asked to report to the exercise science research center for exercise trials. They were instructed to collect 24- hour urine samples along with a separate first- morning void and bring a completed diet log. An extra 16 ounces of water was consumed before going to sleep and upon waking the day of the trial to ensure they would arrive in a euhydrated state.

Prior to entering the environmental chamber, the participant was instrumented with physiological monitoring devices (rectal thermometer for temperature assessment and attached a heart rate monitor). Subjects wore work attire that included gloves, a hard hat, and fire resistant cotton overalls. Prior to work activities, the participant was seated for 10 minutes. After ten minutes, the work protocol began, this which included treadmill walking, lunges with rotations, lifting and carrying 30-lb boxes, carrying 5-lb dumbbell weights over stairs (one in each hand), and tightening and loosening nuts and bolts on a plywood board. The exercises were completed in 45 minutes followed by a 15-minute rest. During the rest, the participant received 750 ml of fluid to drink. The participant was then asked to repeat the 45-minute work in the heat for a second round. Following the second round, they recovered for another 15 minutes.

Post- workout, the participant was asked to leave the environmental chamber and provide a small spot urine sample. Their body mass was taken, and 110% of their body mass lost or 1 L was provided. They were advised to finish their provided drink within 2 hours, and to not drink any other fluid within 3 hours post-trial. They were provided with another 24-hour diet log, 3 urine containers (one for urine sample at 3 hours post-trial, one for the 1st morning sample, and one for 16-hour collection). At the third hour post trial, they were instructed to urinate in the 3-hour post-trial container. After this void, they were told to urinate in the 16-hour collection container. They were allowed to drink whatever they preferred after the trial was completed. The

next morning, they collected their first morning sample and all other voids were placed in the 16-hour container. They were then scheduled to return to the lab ~16 hours following their trial.

On the third visit, 16 hours post-trial, the participant provided their diet log and urine samples. A small spot urine sample was also collected upon arrival.

Urine data analysis

The urine was collected to measure its plasma osmolality and USG. The test for plasma osmolality was completed twice, and not done a third time unless the difference in the two values were >5 $mOsm \cdot kg^{-1}$. The USG was measured and should range from 1.002 to 1.030 (Stuempfle and Drury 2003). All results were recorded for comparison of measurements.

Statistical Analysis

We analyzed the data by running a variety of reliability and validity statistics between hydration assessments. In determining reliability, we computed Pearson r correlations between variables at each time point. To test validity, we compared data at each time point using paired-samples t-tests. Further, to evaluate clinical relevance of differences in categorization of hydration status, we examined the data using Bland-Altman plots.

Chapter IV: Results

The mean urine specific gravity for all pre-trial 24-hour samples was 1.013 ± .005 and the mean osmolality for all 24-hour samples was 483 ± 190. The USG mean for all the pre-trial spot samples was 1.01 ± .006 and the mean osmolality was 500 ± 243. The mean urine specific gravity for all the pre-trial first-morning samples was 1.02 ± .005 and the mean osmolality for the pre-trial first-morning samples was 637 ± 193. For all the post-trial 16-hour urine samples,

the mean USG was 1.02 ± .007 and the mean osmolality for the 16-hour urine samples was 719 ± 221. The mean urine specific gravity of all the post-trial spot samples was 1.02 ± .007 and the post-trial spot mean osmolality was 636 ± 220. The post-trial first-morning samples had a mean urine specific gravity of 1.01 ± .005 and the mean osmolality for the post-trial first-morning samples was 435 ± 200.

Figures 1-8 represent the data points collected during the study related to their standard deviations of 1.96 and the clinical acceptance values. Table 1 shown below represents all the data collected in relation to the results of the specific tests conducted.

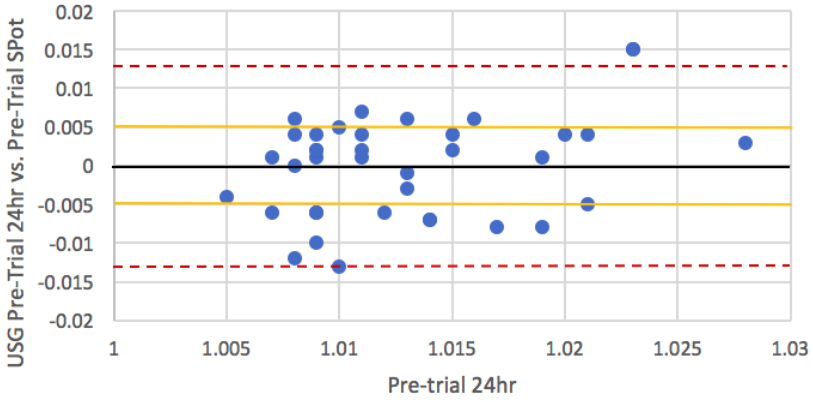


Figure 1.

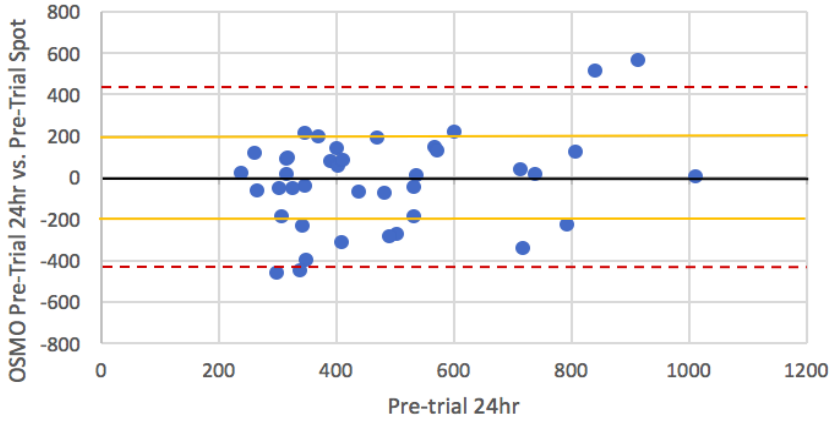


Figure 2.

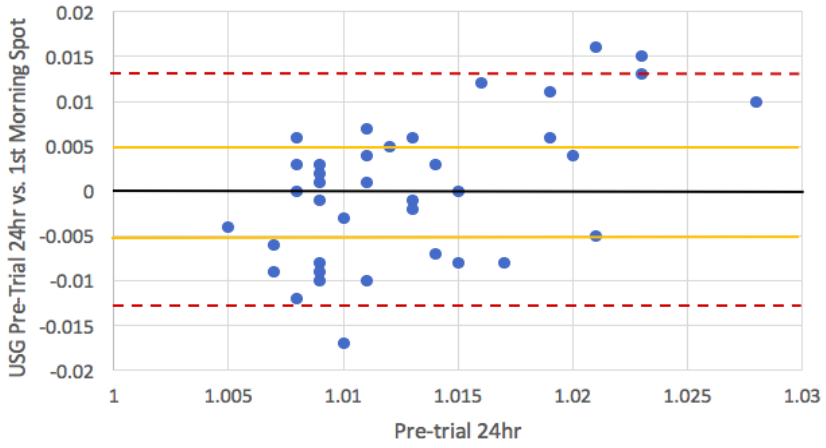
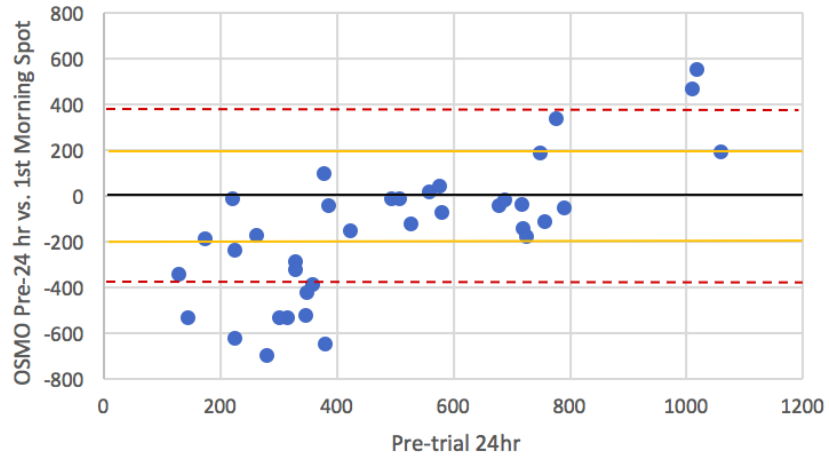


Figure 3.



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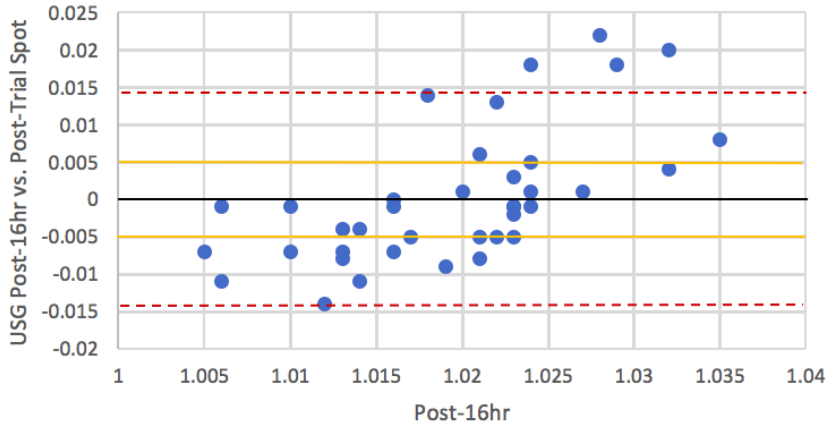

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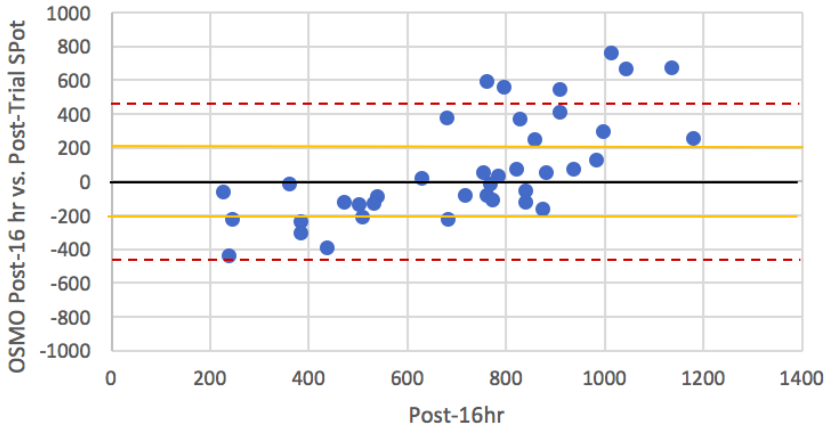

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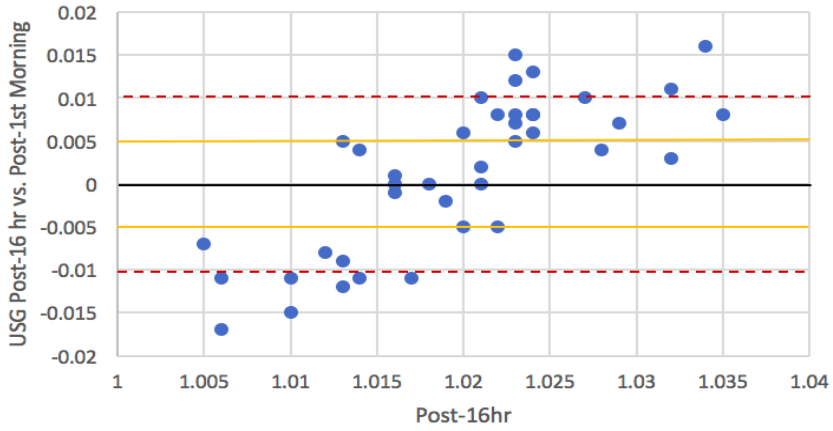

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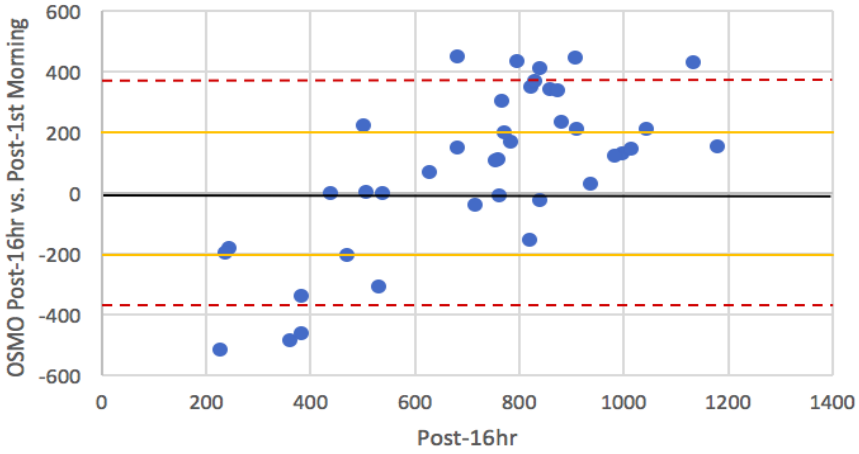

Figure 8.

Table1. Tests conducted for Bland-Altman data points

Trials	Mean Difference (mosm/L)	Paired Samples T-Test	Pearson r correlation	Diagnostic Inaccuracy
USG Pre-Trial 24hr vs Pre- Trial Spot	-0.0000789±0.0066	p=0.941	r=0.468 ; p=0.003	42%
OSMO Pre-Trial 24hr vs. Pre-Trial Spot	-16.48±226.5	p=0.648	r=0.501 ; p=0.001	28%
USG Pre- Trial 24hr vs. 1st Morning Spot	-0.00562±0.006	p<0.001	r=0.358 ; p=0.030	53%
OSMO Pre- Trial 24hr vs. 1st Morning Spot	-154.75±243.9	p<0.001	r=0.229 ; p=0.154	40%
USG Post- 16hr vs. Post- Trial Spot	-0.0079±0.0098	p<0.001	r=-0.124; p=0.459	45%
OSMO Post- 16hr vs. Post- Trial Spot	283.9±305.99	p<0.001	r=0.081 ; p=0.621	45%
USG Post- 16hr vs. Post 1st Morning Spot	-0.00618±.0091	p<0.001	r=-0.250; p=0.130	63%
OSMO Post- 16hr vs. Post 1st Morning Spot	-202.3±249.6	p<0.001	r=0.218 ; p=0.177	48%

Chapter V: Discussion

The purpose of this study was to determine what technique would be an accurate measurement of an individual's hydration status. According to current research on hydration assessment, the "gold standard" to assess the hydration of an individual is by measuring their total body water (Armstrong, Lawrence 2007). This is the most accurate measurement in a laboratory setting, but finding a measurement that works during a real time setting has been a challenge. It was proposed that a spot urine sample would provide more accurate results than a first- morning urine sample when compared to a 24-hour urine sample set as the "gold standard" of this experiment.

Cheuvront claims that a spot urine sample is less accurate than a first- morning void due to several influencing factors (Cheuvront et al., 2015). Based on the data found above, it was concluded that neither a spot urine sample nor a first- morning sample was more accurate than the other. A Pearson r correlation test was completed, and showed that the pre-trial spot sample compared to the pre-24-hour sample was not correlated as well as the first- morning spot sample compared to the pre-24-hour sample. A paired sample t-test was also conducted for both samples. The pre-trial spot sample compared to the pre-trial 24-hour sample showed signs of similar values since the p value was greater than .05 for both USG and osmolality data.

However, when comparing the p value of the first- morning sample to the pre-trial 24- hour sample, the t-test concluded that the values for USG and osmolality were significantly different. Based on the Bland-Altman graphs, both first- morning and pre-trial spot urine samples had little data points outside its standard deviation. In contrast, both samples had a large amount of data points outside the range of clinical acceptance. Therefore, the diagnostic inaccuracy observed shows that a first- morning sample and a spot urine sample are not clinically related to a standard 24-hour urine sample. Contrary to Cheuvront's findings, our data concluded that it is not necessary to use a first- morning sample over a spot urine sample for better accuracy.

A first- morning urine sample combined with body mass is only a "day to day" hydration value, where a spot sample would be more beneficial, if proven accurate, for a hydration assessment before or after activity in an everyday environment. Findings based on Haan's research concluded that a spot urine sample could provide the most accurate results if a steady state was achieved before collecting the urine sample. It was concluded that a spot sample, with tightly controlled fluid and diet intake, was more accurate to the 24-hour urine sample than the first- morning urine sample (Hahn, Waldréus 2013). The morning void was overly concentrated and was not consistent with the 24- hour urine values. The spot urine samples gathered in our study were taken in the afternoon, but unlike in Hann's study, the fluid and diet of the participants was not regulated before the sample was taken. Therefore, a steady state was not met, and this could be why our spot sample did not demonstrate accuracy when compared to the 24- hour urine sample.

Gowans and Frasier stated that there was a significant difference in values when comparing a spot urine sample to a 24- hour sample, and little difference between the first-morning void and the 24- hour urine sample (Gowans, Frasier 1987). Based on our data, both

values were significantly different to the 24-hour standard sample used. Gowans and Frasier may not have used a regulated steady state for their spot sample which would have provided a weak correlation to the 24-hour sample. The first- morning sample in our study showed more negative osmolality points on the Bland Altman graph, which could have been due to an overly concentrated sample. Bottin claims that over concentrated morning samples can be caused due to the body fasting from food or water during sleep (Bottin et al., 2016). This could be why our first- morning urine sample was inconsistent with the 24- hour urine sample.

In Perrier's study, afternoon spot samples were more consistent with 24- hour urine samples than a first- morning sample. It was hypothesized that a spot sample could even replace a 24- hour urine sample for testing purposes. It was concluded that the first- morning samples were overly concentrated compared to the 24- hour sample (Perrier et al., 2013). Based on the data found in our hydration study, the spot samples were taken in the afternoon, yet were still significantly different to the 24- hour urine sample. The spot sample was shown to be similar to the 24- hour values in the paired sample t-test conducted. Yet, due to the lack of correlation in the Pearson r correlation test and the diagnostic inaccuracy, this only shows the values are statistically similar but may not be accurate to use. If the diet and water intake of the participant was controlled, the results could have shown more accuracy based on what other studies have shown.

Along with the 24-hour urine sample comparisons, a 16-hour urine sample was also used in comparison with the spot sample and first-morning sample post-exercise trial. A limitation to the analysis is that the 16-hour urine sample has not been validated in previous research. The sample only contains a portion of the day along with the individuals sleep period, where the body is fluid fasted. Yet, within such a large study, this was the data that was available to use for the

purpose of this specific research question. The 16-hour sample included all urine output after exercise, as well as the first-morning and spot sample. In a clinical setting, different urine samples can be obtained at different time points other than a first-morning or spot sample. Therefore, the 16-hour urine sample was still chosen for comparison. When comparing the first-morning and spot samples to the 16-hour urine sample, the same relationship that was seen within the 24-hour sample comparisons existed. Neither the first-morning or spot sample was more accurate than the other when compared to the 16-hour urine sample.

A relationship was observed to the concentration of urine and the individuals 24-hour hydration levels. . It was observed that when the participant was hydrated throughout the 24-hour period prior to exercise, their first-morning urine sample was over concentrated as shown in Figure 4. This was due to a steady state achieved overnight. This caused the first-morning urine sample to be over concentrated as expected.

In contrast, when the individual was dehydrated, the first-morning urine sample was diluted. Perhaps these individuals drink very little during the day and are not well hydrated over a 24-hour period. All participants were instructed to drink an excess amount of fluid the night before their trial to ensure they were in a state of euhydration. Therefore, the first- morning sample was shown to be diluted, skewing the results. This is an example of how an influx in fluid can influence urine sample results if a steady state is not produced prior to collecting the sample. If they would not have been prescribed to drink extra fluids prior to exercise, their samples would have most likely indicated they were still dehydrated. In a clinical setting, for someone who is a low drinker or chronically dehydrated, their urine sample would not be indicative of their correct hydration status unless a steady state was produced.

Chapter VI: Conclusion

In conclusion, it has been debated as to what form of hydration assessment will provide the most accurate results. Based on our data, both the first- morning urine sample and the spot urine sample when compared to the 24- hour urine sample exhibited diagnostic inaccuracy. If a pre-trial spot sample was used to assess hydration using both USG and osmolality, the average diagnostic inaccuracy would be 35%. If using the USG and osmolality of a pre-trial first-morning sample compared to the 24-hour urine sample, the patient's hydration status would be misdiagnosed on average 47% of the time. The post-trial urine samples obtained showed an even larger range of inaccuracy. The spot sample's clinical inaccuracy increased to an average of 45%, and the first-morning sample increased to about 56%. After statistical analysis, the Bland-Altman plots show that neither the first- morning sample or the spot sample were more clinically accurate than the other, as they both showed a large amount of data outside the accepted diagnostic values. Therefore, for future research, it may be beneficial to use a 24-hour urine sample when conducting hydration assessment, if possible. If a 24- hour urine sample is unattainable, either a first- morning sample or a spot urine sample could be used because neither are significant different from each other. It is important to note, whichever sample is chosen should be consistent throughout data collection.

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