Urine Refractometry to Gauge Hydration Status in Recreational Female Runners

A Senior Honors Thesis

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ABSTRACT

Urine specific gravity (USG) using refractometry has been reported to be an accurate and sensitive method of evaluating hydration status in various models. However, little evidence using USG is documented on recreational female runner subjects. The purpose of this study was to assess the validity of the refractometer as a pre-screening tool to assess hydration status before drawing blood samples to avoid drawing dehydrated blood in a larger parent study designed to evaluate the female athlete triad in adult female recreational runners. We obtained 124 blood samples from the 125 consented subjects. Blood was drawn only if USG analysis indicated euhydration (USG <1.020). The Ohio State Medical Center Laboratory defined hydrated blood osmolality at 275-295osm/kg. The mean USG of the population was 1.008 (SD= 0.005), indicating a well-hydrated group of female runners. We used correlation and logistic regression analyses to investigate relationships between USG and blood osmometry values. There were no correlations or significant relationships between USG and osmometry whether evaluated as continuous or categorical variables. The lack of relationship may be the result of ad libitum fluid consumption prior to the lab visit resulting in a few overly hydrated subjects. Further studies on the design of the pre-visit fluid consumption protocol might reveal stronger relationships. However, from this study, it was concluded that refractometry was a successful pre-screening measurement to avoid drawing dehydrated blood in recreational female runners, as none of subjects had dehydrated blood values (>295osm/kg).

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CHAPTER 1: INTRODUCTION

Hydration status of athletes can either help to maximize performance or diminish performance and hold health consequences in both competition and training.[20] Therefore, convenient estimation of hydration status is an important barometer for athletes to help avoid dehydration related health complications, including heart syncope, heat exhaustion, and even death.[2,3] Current literature suggests that urine specific gravity (USG) is a sensible and a valid measurement for assessing hydration status in athletes.[5, 20, 21, 28]

There are other situations when it is desirable to non-invasively gauge hydration status. Evaluation of blood values in a dehydrated state will likely lead to falsely elevated or depressed serological measures for the variables of interest, thus being able to accurately screen for hydration prior to drawing the blood would be an advantageous screening tool. Once blood has been drawn, blood osmolality (moles of solute/kilogram of solvent) is typically the common marker to ensure adequate hydration status, alongside the measures of interest. However, once the blood sample has been obtained and analyzed for osmometry and the substances of interest, it is too late to find out the samples were dehydrated. A non-invasive simple technique that would foreshadow existing dehydration would be useful in clinical and research studies. In an effort to decrease the amount of hypohydration-related health issues and to save on the costs of blood osmolality analysis, more research is needed to assess the validity of methods, such as the USG as a screening tool for hydration status.[5]

CHAPTER 2: HYPOTHESIS

The current study evaluated the correlation between urine specific gravity levels and osmolality of blood in recreational female runner subjects. The purpose of the study was to assess hydration status during a routine research laboratory visit using refractometry as the urine specific gravity method and laboratory evaluation of serum osmometry. Using refractometry, we predicted that USG values would strongly correlate with blood osmolality. The underlying motivation for screening the urine was to avoid having blood results indicating high osmolality (dehydration), thus negating the results of other markers of interest and necessitating an additional blood draw and cost.

Null Hypothesis: There is no significant correlation between USG and blood osmolality.

Alternative Hypothesis: There is a strong and significant correlation between USG and blood osmolality.

CHAPTER 3: REVIEW OF LITERATURE

INTRODUCTION

Urine specific gravity has been used in various models for urine analysis. For example, Dossin et al. compared three methods of assessing urine specific gravity in dogs.[11] These methods included total body weighing, refractometry, and reagent strips. It was determined that refractometry was the superior method for evaluating urine concentration (hydration) among these three methods in dogs. White et al. evaluated USG levels in gorillas and woolly monkeys to determine hormone concentrations, especially cortisol, in the urine.[33] The study suggested that refractometry USG values were strongly correlated with creatinine-corrected cortisol values. Therefore, it was suggested that refractometry (USG) was a valid technique for studying physiological conditions of unrestrained animals. Various animal models have demonstrated the validity of USG by refractometry.

The literature evaluation of USG is not limited to animals as studies have looked at USG analysis in humans as well. In particular, researchers have been drawn to USG as an indicator of hydration status in athletes. As athletes partake in strenuous exercise, testing for adequate hydration in a non-invasive, relatively cheap manner becomes an important issue to prevent health risks associated with dehydration. For example, in an effort to decrease the amount of dehydration-related health issues among collegiate athletes, the NCAA has implemented the use of urine specific gravity into the regulations for the weight-certification process to ensure adequate hydration of wrestlers.[19] Because it has been common to control weight through

water loss, it is no surprise that much of the assessment of hydration status centers on wrestlers for safety purposes.

Bartok et al. have multiple studies evaluating refractometry as a means for urine analysis in exercising populations.[5] In addition, other studies examined the use of the urine refractometer to measure urine specific gravity in athletes and recreational exercisers, and conclude USG to provide a robust and objective measure of hydration in similar populations.[4, 28] However, the research literature provides scant studies on recreational female runners.

ETIOLOGY OF DEHYDRATION

The American Medical Association defines hypertonic dehydration as reduced plasma volume or hyperosmolarity of the blood (>300mmol/kg) which can be exercise-induced.[31] Severe reductions in weight during an acute exercise bout are reflective of a significant change in the athlete's hydration status, as the extra weight loss is due to increased sweat loss and the lack of hydration during training.[5, 25] Dehydration in athletes is usually associated with sweat loss, thus also associated with environmental extremes and the lack of appropriate fluid and electrolyte consumption. Dehydration has been shown to decrease performance, and mild dehydration can progress to serious life-threatening situations.[31] In extreme situations, reduced body-water, or hypohydration, is followed by a spike in core body temperature, an increased heart rate to compensate for decreased stroke volume and potential hypernatremia.[9] "Furthermore, insufficient fluid intake alters cellular functions including metabolism, excitation, hormone release, cell proliferation, and cell death."[4] Therefore, athlete hydration status

undoubtedly affects performance and health-related risks, which prioritizes an accurate non-invasive measurement technique.

MEASUREMENT OF HYDRATION STATUS

Blood osmolality is considered the most common clinical measurement of hydration status but other less invasive measurements that rely on the concentration of urine are commonly used in practice.[10, 11] Shirreffs even suggests that urinary measurements including urine specific gravity are more sensitive methods for gauging hydration status than blood indices.[26] Blood and urine are common indicators for hydration levels, and there is some debate about which is the better measure.

Other methods have been considered in research for assessing hydration status. Gravimetry is a direct measure of urine specific gravity, whereas refractometry and reagent strips are considered indirect measurements.[10] However, in 1999, the NCAA removed the use of reagent strips as an estimate of hydration status in athletes due to the perceived inaccuracy of reagent strips when compared with refractometry.[19] This was partly due to the death of three wrestlers in 1997, which caused more concern for wrestlers losing too much water weight and increased the safety focus on hydration status.[23] In addition, previous literature from 1983 demonstrated that reagent strips were not adequate for clinical use when compared to refractometry.[1] Stuempfle also noted from her study on collegiate wrestling athletes that only refractometry was valid to determine USG.[29] Gravimetry and urine reagent strips have also been considered for urine evaluation of hydration, but the currently accepted method is the urine refractometer.

Refractometry is an index that measures the "ratio of the velocity of light in a vacuum to the velocity of light in a solution."[32] It is considered valid and reliable[19] and potentially a more sensitive approach than using plasma osmolality according to Armstrong.[2, 3, 17] Literature suggests that refractometry is valid when compared to hydrometry.[18] In Stuempfle's study concerning the reliability of refractometry, hydrometry and reagent strips were discussed, as was the validity of refractometry. From this study, it was concluded that refractometry and hydrometry were reliable between trials for each tester. Reagent strips did not prove to be reliable between trials of wrestling athletes. However, none of the subjects were dehydrated at the onset so reliability was tested only on euhydrated subjects, which limits the results to euhydrated subjects only.[29]

Popowski et al. simultaneously evaluated USG and osmolality levels of urine for correlation with osmolality of the blood using refractometry and reagent strips.[22] This study observed physically fit males with a mean weight of 73.4 kg as they lost body mass via dehydration.[22] Both USG and blood osmolality were measured at 1%, 3% and 5% dehydration and at a 1-hour of rehydration to compare blood osmolality and USG sensitivities. The study determined a moderate correlation between blood osmolality and USG ($r=0.46$, $p>0.10$).[19, 22] Popowski et al. suggested these strips were strongly correlated to refractometry.[22] Popowski's study took place after the adoption of the NCAA guidelines determined reagent strips did not present strong enough evidence to be included in the wrestling weight certification program. Popowski et al.'s results concerning reagent strips contradicted the NCAA guidelines, which presents the case that even though the reagent strips were banned for wrestling athletes, their

validity is contradictory. Refractometry is more consistent in the literature as a more valid method.

Similarly, Lew et al. used refractometry to measure hydration status in male and female Singaporean athletes who underwent body mass reductions through fluid loss. It was suggested that USG levels increased by ~0.003 units with every 1% body mass reduction when exercising in an environment that induced dehydration.[16] Lew et al. suggested that USG via refractometry is sensitive enough in detecting fluid deficits. However, since USG measurements were only measured at 1% body mass reductions each day for five days, the authors noted the results were not as conclusive as the Popowski study previously discussed.

HYDRATION STATUS THRESHOLDS

Various organizations have published guidelines to define hydration status. The National Athletic Training Association (NATA) guidelines define a well-hydrated status at a USG levels <1.010; minimum dehydration between 1.010-1.020, significant dehydration between 1.021-1.030, and any USG value above 1.030 as serious dehydration.[8, 9] Slightly different from the NATA guidelines, the National Collegiate Athletic Association (NCAA) has accepted a USG value of 1.020 as the cusp between euhydration and "hypohydration" in athletes, with any value below 1.020 as euhydration and any USG value above 1.020 as dehydration.[19] The American College of Sports Medicine also provides a guidance document defining <1.020 as euhydrated with two levels of dehydration defined.[25] The NATA, ACSM and NCAA are the three governing bodies most likely to define guidelines for athlete hydration status.[8, 19, 25] Table 1 compiles the various guidelines for a more simplistic view.

Table 1: Values adopted by professional organizations: National Collegiate Athletic Association (NCAA, National Athletic Training Association (NATA,) American College of Sports Medicine (ACSM) and Armstrong's Method (each category represents a percentile of the population)

Hydration thresholds:	NCAA	NATA	ACSM	Armstrong Method #1	Armstrong Method #2
Extremely Dehydrated	-	>1.030 (serious)	-	-	91-100% of population (~10%)
Very dehydrated	-	1.021-1.030 (significant)	> or = to 1.030	-	76-90% of population (~15%)
Minimal/slight Dehydration	1.020-1.025	1.010-1020	-	-	61-75% of population (~15%)
Hypohydration	>1.020	-	1.020-1.029	1 standard deviation > mean	-
Euhydration	-	<1.010	<1.020	Mean of population	41-60% (middle 20% of population, mean)
Well-Hydrated	<1.020	-	-	1 standard deviation < mean	26-40% of population (~15%)
Slightly Hyperhydrated	-	-	-	-	11-25% of population (~15%)
Extremely Hyperhydrated	-	-	-		1-10% of population (10%)

Bartok et al. also advocated for 1.020 as an accepted threshold of euhydration in athletes.[5] In their study, wrestlers with a mean body mass of 81.7kg were measured in euhydrated and dehydrated states in the same day. These wrestlers lost 2-6% body mass via exercising in a hot environment along with fluid restrictions. As a response to dehydration, urine specific gravity levels increased as the wrestlers became more dehydrated. Bartok et al.

concluded that 1.020 was a valid cutoff using the Receiver Operator Characteristic (ROC), which is a measure of the area under the curve. ROC analyses distinguish between false- positives and true-positives and find an appropriate trade-off of false-positives versus false-negatives.[15] Using 95% confidence intervals; Bartok et al. used the cutoff that showed the lowest false negative and false positive values. 1.020 had a specificity (true negative rate) and sensitivity (true-positive rate) of 96%. Thus, based on low numbers of false positives and false negatives, these researchers agreed with the NCAA threshold of 1.020 as the cutoff USG for collegiate wrestlers.[5]

Armstrong et al. employed a different method for determining the USG cutoff points to define dehydration. These researchers suggested the mean USG and standard deviation values among a group of athletes should be used to define the threshold for euhydration.[3] In this model, well-hydrated was defined as one standard deviation less than the mean USG value of a group and slightly dehydrated is one standard deviation above the mean USG level. The mean of the sample represents the center of the euhydrated category. Stover et al. used this Armstrong method in their study on recreational exercisers. Adequate hydration was defined as 1.011 to 1.025 with a mean of 1.018 for their group of recreational exercisers.[28] The cutoff for being well-hydrated was determined to be <1.011 and hypohydration for recreational exercisers was >1.025. The ultimate purpose evaluating these cutoff values was to observe the correlation between USG and blood osmolality in free-living exercisers. It was concluded that using refractometry to measure USG strongly correlated with blood osmolality values. (r=0.995, p not reported).[28] Stover is a strong proponent of using urine refractometry.

Later research by Armstrong et al. suggested a different method based on percentiles to establish a range of hydration from extremely hyperhydrated USG values to extremely dehydrated USG values [4] They used a group of 59 healthy men (mean weight=75.1kg) to determine USG values from an initial morning sample and from a 24-hour sample. Each extreme of the hydration spectrum represented 10% of the 59 men studied (ie, hyperhydration was the lowest 10 percentile and dehydration was the highest 10 percentile). Euhydration, which was the central category, was considered to be the middle 20% of the population. Specifically, Armstrong et al. determined that USG values <1.017 or <1.012 represented extremely hyperhydrated in morning and 24 hour samples, respectively. These authors obtained urine samples over a 12-day period, where it was determined that the mean euhydration range for this group was 1.018-1.020 for 24-hour samples and 1.024-1.026 for initial morning samples. The study concluded that the mean USG for each day was consistent among the 12 days for both morning and 24-hour urine samples. The researchers commented that due to decreased glomerular filtration at night, it was not surprising the morning samples proved to be more concentrated than 24-hour urine samples.[4]

When comparing the works of Armstrong and Stover, there is little discrepancy in USG thresholds. Armstrong et al. determined the mean USG was >1.018, or 1.018-1.020 (24-hour) and 1.024-1.026 (morning sample) with a standard deviation of 0.006 in active men. Stover et al. determined a mean USG of 1.018 with a standard deviation of 0.007 and 0.008 for men and women recreational exercisers, respectively. Stover et al. ultimately suggested using 1.025 as the cutoff for recreational exercisers. Any discrepancy in terms of defining euhydration for athletes versus recreational exercisers may be attributed to athletes performing more

strenuous training and generally sweating more than recreational exercisers. Thus, the value 1.020 as the upper USG threshold for euhydration is more widely used among athletes.[28] Using 1.025 as the cutoff for free-living exercisers relies on the assumption that these recreational exercisers were not as hypohydrated as athletes undergoing severe training. Stover demonstrated 46% of the free-living exercisers were considered to by hypohydrated.[28] In contrast, Volpe et al. studied a population of male and female athletes and concluded 66% of these athletes were dehydrated.[30] However, Stover et al. suggested that more research is needed using USG levels as a hydration indicator in exercise-induced dehydrated subjects.[28] Despite the evidence that suggests somewhat similar values for the thresholds between hypohydration and euhydration, further research is needed to examine the extremes of the hydration spectrum including hyperhydration. Thus far, the NCAA and the NATA do not evaluate hyperhydration with a separate cutoff.

TRENDS OF HYDRATION STATUS

One trend observed in recreational exercisers was that, on average, men had a higher USG specific gravity than women, indicating more men were in the hypohydrated category than women.[28] This was consistent with Volpe's study, which observed the hydration status of athletes, where women athletes tended to also be better hydrated according to urine values.[30] One suggested explanation offered by Volpe was that women have a higher thermoregulatory threshold, which delays onset of sweating until a higher core temperature is reached.[30] In addition, ACSM suggested that females retain more water in the body than men because of

lower sweat rates.[25] The literature does support a gender difference in defining hydration status according to urine refractometry.

A study designed by Bossingham et al. also looked at the difference in urine specific gravity between men and women independent of age.[7] Despite having a controlled diet with controlled amounts of protein, men had a significantly higher USG than women ($p<0.001$). In contrast, the blood osmolality was not shown to be significantly different between men and women. However, since this study controlled the diets for men and women, the variation of protein intake and other dietary components could explain the difference in USG.[7] Although current literature does not suggest a concrete explanation for the difference in gender USG, future research should account for this difference that women are typically better hydrated than men when using urine analysis.

There is contradictory information in the literature regarding the time of urine sample. On one hand, there was no significant difference between collecting urine samples in the morning or in the collecting the samples throughout the day when comparing hydration status among the athletes.[3, 30] On the other hand, hyperhydration had a very low prevalence in morning samples in Armstrong's study.[4] These morning samples were more concentrated, as there was little fluid intake in the morning among these athletes.[4] Time of day and activities before sampling is likely an important consideration when designing studies of hydration status.

FREE WATER CLEARANCE DURING REHYDRATION

Bodonyi-Kovacs described free water clearance as an excess of free water loss. As most electrolytes, mainly sodium and potassium are reabsorbed, free water clearance might be a

better reflection of blood sodium and potassium levels rather than a reflection of hydration status.[6] As the body becomes dehydrated, there is a loss of water as well as sodium and potassium. During rehydration from an acute dehydration bout, the body strives to regain fluid and electrolyte homeostasis. In the blood, this means having a physiological concentration of sodium and potassium. When the fluid consumed is more dilute than the blood (hypotonic), the kidneys will excrete the extra fluid to avoid a further depression of the electrolytes. Conversely, when hypertonic fluids are consumed, it may help the body retain the fluids as it strives to reach homeostasis. When a dehydrated person consumes a hypotonic fluid like water, it might result in free water clearance where the blood and urine osmolality would not necessarily be reflective of each other until homeostasis is reached.

Evans et al. studied the efficacy of hypertonic glucose solutions' as a post-exercise rehydration fluid in adult males where the sodium in the fluids was the same (25mmol/L). To do this, they evaluated free water clearance (volume of urine production) at each hour of recovery for four hours using the equation $FWC=V(1-U_{osm}/S_{osm})$ (V=urinary flow rate, U_{osm}=urine osmolarity, S_{osm}=blood osmolarity). Exercisers ingested 0%, 2%, or 10% glucose solutions 30 minutes after an acute dehydration bout. Blood and urine indices were tested periodically for up to 6 hours. It was concluded that there was a greater free water clearance among the 0% glucose trial compared to the 10% glucose trial.[12] After one hour of rehydration, the 10% glucose solution was better retained. It was noted that a more hypertonic solution such as the 10% glucose solution delayed gastric emptying, and plasma volume was maintained in addition to plasma sodium concentrations. Ultimately, ingesting the hypertonic solution resulted in smaller amounts of fluid loss, which allowed the subjects to return to an appropriate hydrated

state in a timelier manner. Additionally, the 2% glucose solutions tended to have a greater free water clearance than the more hypertonic 10% glucose trial, as hypotonic solutions promote the loss of free water. This study suggested, "Hypertonic glucose-sodium drinks may be more effective at restoring and maintaining hydration status after sweat loss than more dilute solutions when the sodium concentration is comparable."[12] The 10% glucose solution allowed subjects to remain in a euhydrated state for a longer period of time post-rehydration period than the 2% solution.[12] Although there were significant differences in urine volume among the three glucose solutions at one-hour post-rehydration, the retention of the ingested fluid was similar among the 0%, 2%, and 10% glucose solutions at 6 hours post-rehydration. These results might hold the largest impact for multiple session or event days.

Similarly, Shireffs et al. looked at the sensitivity of plasma changes during a recovery period post-exercise.[27] Plasma osmolality was assessed pre-exercise to confirm that the subjects were all in a normal range of hydration (283-293mosm/kg). Dehydration levels were attained through immersion in a water bath for 10 minutes followed by 60 min of exercise or until 2% body mass was lost. 12 healthy male subjects were initiated into an incomplete randomized four-way crossover design. Fifteen minutes post-exercise, the subjects ingested 50%, 100%, 150% or 200% the volume of fluids lost during exercise. Sodium concentration was also variable in the study as subjects consumed 2, 20, 50, or 100 mmol fluids relative to sodium content. It was concluded that in order to properly restore plasma volume levels, both sodium concentration and fluid volume were factors to consider for restoration of pre-exercise body weight. Fluid volume consumed should be more than fluid loss, but in addition, sodium

concentration should be close to the concentration of sweat in order to restore and maintain electrolyte concentrations in the blood.

As previously discussed, Popowski et al. measured USG in subjects who underwent a 1%, 3%, and 5% body mass reductions via sweat loss.[22] The results supported the conclusion that measurement of blood osmolality was more responsive than urine in the immediate hour of rehydration recovery. The authors concluded that urine indices of dehydration have a lag time when compared to the more immediate detection using blood osmolality. The subjects in Popowski's study ingested fluids during a 1-hr rehydration period to restore fluid losses from a 5% body weight reduction. The researchers concluded; however, that despite the equal ingestion of fluid, a one-hour period was not enough time for the re-establishment of body weight after 5% dehydration. This study indicates that the time period necessary to normalize various levels of dehydration may impact measures of refractometry or osmometry recorded around the dehydration episode.

SENSITIVITY AND SPECIFICITY

False positive (specificity) and false negative (sensitivity) tests for dehydration have also been considered in the conversation for dehydration screening using USG and serum osmolality.[22] A false negative, as defined by Popowski et al., included a subject who was detected as dehydrated with a USG greater than 1.020, but was considered euhydrated using blood osmolality. A false positive test consisted of just the opposite, where the subject was euhydrated with urinary USG values, but considered dehydrated based on blood osmolality. Earlier research by Francesconi et al. indicated that hypohydration (USG >1.030) was distinctly

present in urine samples that were concurrently elevated in creatinine in subjects with 3% body mass loss due to dehydration.[13] Despite these urine indices, hypohydration was not necessarily indicated in blood osmolality.

Hamouti et al. conducted a study that focused on comparing rugby players' hydration status to runners.[14] It was concluded that rugby players on average, had a higher USG than runners, but the electrolyte concentrations among the two groups' urine samples were not significantly different. As a result, this study suggested that larger muscle masses in these rugby players might be responsible for hypohydration USG >1.020. The specificity of hydration evaluation in rugby players was found to indicate that these athletes with larger muscle masses were more "dehydrated" when using USG, but the athletes were adequately hydrated when using plasma osmolality as an indicator. Positive correlations were found with metabolites in urine and muscle mass, thus Hamouti defined the USG results as false positives in favor of defining the hydration status more accurately with blood osmometry. It would be simpler to define dehydration if urine and blood outcomes matched more closely. The equivocal results challenge the researcher to decide which measures to take and at what point in the protocol.

CHAPTER 4: METHODS

Recreational female runner subjects were recruited for a parent study on the female athlete triad, and the hydration data were collected during a routine laboratory visit at Labs in Life @COSI. Subjects who were running 15 miles per week for the past 6 weeks were recruited to the study and screened for exclusion due to thyroid or bone disease as well as medications known to affect bone metabolism. Subjects signed an informed consent prior to participation in the study, and all methods in the study were approved by the Human Subjects Biomedical IRB (Protocol #2009H0177). The parent study consented 125 female runners. Prior to the lab visit, subjects were encouraged via e-mail to be attentive to hydration status by the following statement within a larger instructional e-mail:

> "We do want you to be normally hydrated so drinking enough water the evening before and a glass when you get up would be good."

There were no additional instructions or limits related to drinking water the morning of the study. Subjects were not required to be fasting but were asked not to consume a large meal prior to the study. Immediately after consent and confirmation of study eligibility, subjects were asked to provide a small urine sample. The sample was examined using the Fisher Scientific handheld 13-946-35 Urine Refractometer. Four to five drops of urine were placed on the stage of the tool using a disposable sterile pipette. The refractometer stage is held up towards the light as if viewing the light in a telescope. The button for the internal tool light is depressed to reveal the USG scale where the point of contrast is the USG value. The value for the sample was recorded in g/mm on the subject's datasheet and later entered into Excel.

If the urine USG was <1.020, the subject was seated at the blood draw station and prepared for venipuncture. This included identifying the target vein, cleaning the area, applying the rubber tourniquet, and using a 21 gauge Beckson-Dickson vacutainer needle system to obtain venous blood samples. The blood was centrifuged in the serum separator tube within 15 minutes and placed in the lab refrigerator until transport. Blood for each lab morning was transported to the Ohio State University Hospital laboratory for standard analysis of osmometry and other substances of interest. Results were returned to the research lab via fax and manually recorded on the Excel datasheet for analysis. All data were checked by a different research assistant than the person entering the data to ensure data quality.

Collated data were imported into SAS, version 9.2 for data analysis.[24] Descriptive variables of interest included subject age, height, weight, mileage, and years run. Hydration variables of interest included the USG and blood osmometry values. All variables were examined using the SAS Proc Univariate procedure for normality of distribution. The apriori threshold of $p>0.20$ was set for the Shapiro-Wilk statistic in this determination. Simple correlation and plots using the Proc Corr command examined the linear relationship of USG and osmometry as continuous variables using the Pearson correlation.

Due to hyperhydration of some of the subjects when blood values were returned, the data were then examined using logistic regression to determine if USG (as a continuous variable) had any predictive value for the hyperhydration condition as determined by blood osmometry less than 275 mosm/kg. USG values were categorized using the method of Armstrong where the mean for the sample served as the center of hydration and hyper and

hypohydration were determined using one standard deviation below and above the mean. In this case, the USG was examined as the dichotomous variable using logistic regression where blood osmometry was the continuous predictor variable. In addition, this dichotomous Armstrong variable was compared with the dichotomous osmometry variable using the Chi-square statistic to see if the hyperhydrated and euhydrated states matched according to method. Lastly, the hyperhydrated values were similarly evaluated using Armstrong's percentile method. The lowest 10% of the osmometry values were evaluated to observe any pattern associated with USG. All results are reported.

CHAPTER 5: RESULTS

Blood samples were obtained for 124 of the 125 consented subjects. Urine samples were limited to 108 of the subjects due to the rescheduling procedure when subjects were dehydrated (USG>1.020) at the initial laboratory visit. Data analysis is limited to the subjects with complete hydration data (n=108). Descriptive data for the subjects included in the analysis are included in Table 2.

Table 2: Means of variables in female runner population

Variable:	Mean:	Standard Deviation:	Range:
Age (years)	**34.95**	**10.17**	**18-68**
Miles per week	**22.84**	**9.54**	**10-70**
Years Run	**10.41**	**8.50**	**1-35**

The mean USG for the sample of female runners was 1.008 with a standard deviation of 0.005. This represents a well-hydrated population, as discussed by any of the previous guidelines. The mean blood osmolality was 283.22 (mosm/kg) with a standard deviation of 4.76. While none of the subjects were dehydrated according to blood osmometry, a total of 7 subjects appeared hyperhydrated where osmometry less than 275osm/kg indicated overly hydrated. Both USG and osmometry were normally distributed according to the Shapiro-Wilk statistic (USG p=0.897, blood osmolality p=0.966). Figures 1 and 2 display the normal distribution of USG and osmolality, respectively.

Figure 1: **Blood USG:**

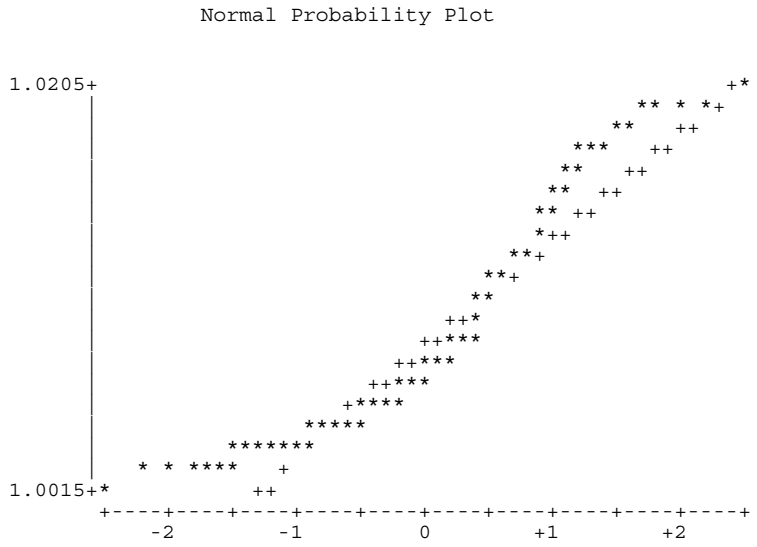
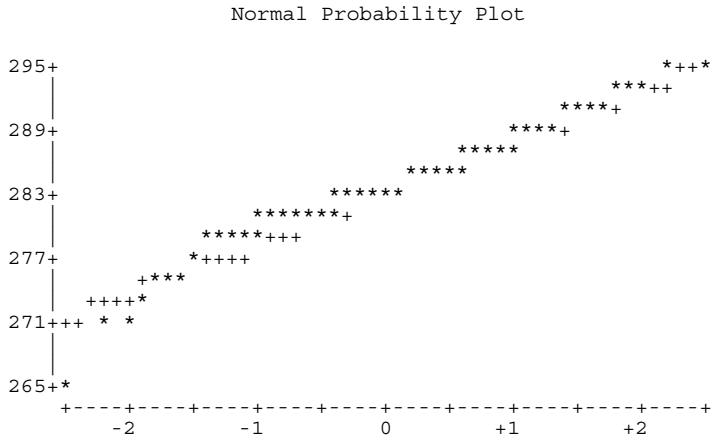


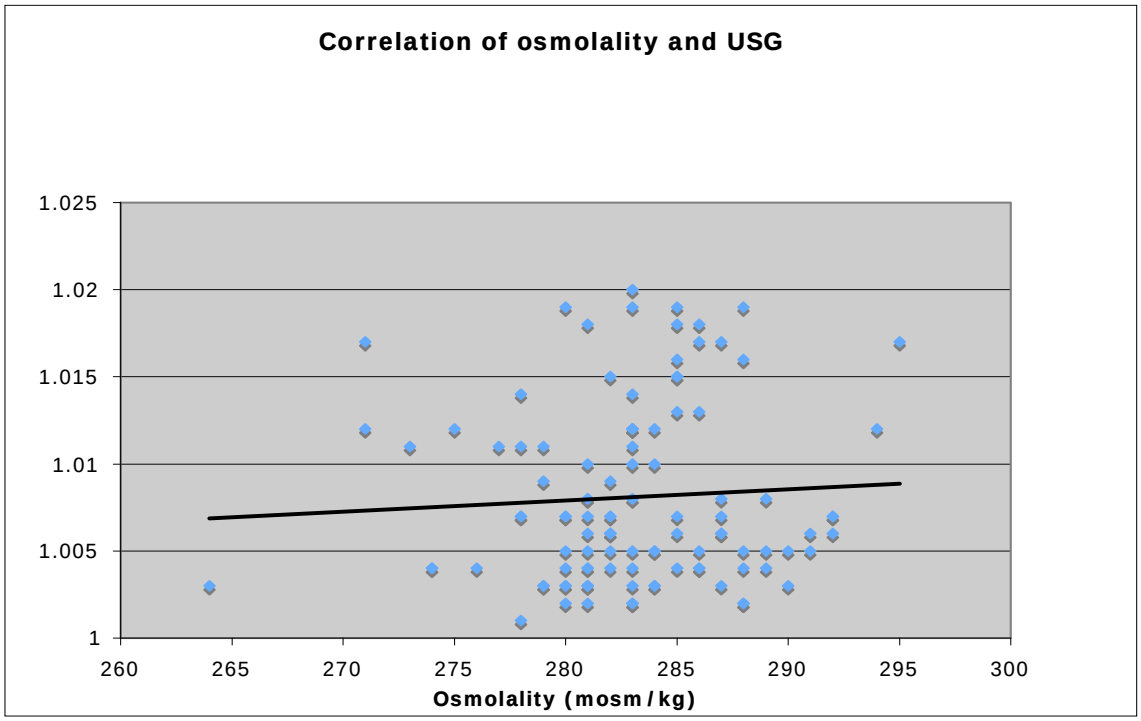
Figure 2: **Osmolality:**



The correlation between the two continuous variables of blood osmolality and USG yielded a Pearson correlation coefficient of r=0.06, with a probability p=0.5296. This indicated no significant correlation between the two variables. This study was unable to compare the relationship of blood osmolality to USG values higher than 1.020 as blood was not taken if USG

indicated dehydration. Figure 3 demonstrates the plotted data for visual examination of the USG and osmolality continuous variables.

Figure 3: Blood Osmolality vs. USG (r=0.06, p=0.5295).



Logistic regression was applied to the data where osmometry was transformed to a dichotomous variable according to the hospital guidelines (hyperhydrated <275osm/kg/euhydrated 275-295osm/kg) to examine the potential utility of a low USG (continuous variable) to predict overhydration. Visual inspections of the hyperhydrated osmometry values did not demonstrate an obvious pattern of USG values. This was confirmed statistically with the logistic regression where results yielded p=0.839.

Logistic regression was also used to evaluate the lowest 10 percentile of osmometry as hyperhydrated in accordance with the research of Armstrong.[4] The lowest ten percent of the group would have been defined by <278 mosm/kg, which would have included two additional subjects with USGs of 1.004 and 1.011 further demonstrating the lack of apparent relationship (p=0.723). Visual examination of the seven lowest blood osmometry values in Table 3 demonstrates the variability of USG values solidifying this conclusion. There was no apparent predictive value of urine refractometry to osmometry status for hyperhydration using this method.

Table 3: Hyperhydrated blood osmolality (mosm/kg) and corresponding Blood USG

Hyperhydrated Blood Osmolality	**Blood USG**
264osm/kg	**1.003**
271osm/kg	**1.012**
271osm/kg	**1.017**
273osm/kg	**1.011**
274osm/kg	**1.004**
274osm/kg	**1.004**
275osm/kg	**1.012**

Logistic regression was also used to see if the sample mean method of categorizing urine specific gravity as put forth by Armstrong had any predictive value. According to this earlier method, the mean USG value of 1.008 would have been the used with the standard deviation of 0.005 to establish the range of adequate hydration. USG values greater than or equal to 1.013 would indicate dehydration, and USG values less than or equal to 1.003 would

indicate hyperhydration. Employing the 1.003 threshold to categorize USG (hyperhydrated or euhydrated) transformed the USG to a dichotomous variable. The transformed variables indicated that 13 subjects had a USG of less than 1.003. A logistic regression was performed to see if the continuous osmometry variable could predict this overhydration. The results yielded a p value of 0.992 indicating no significant relationship. Figure 4 demonstrates a visual display of the sporadic pattern observed when USG values were transformed into this dichotomous variable based on this mean/SD method.

Figure 4: Relationship between hyperhydrated USG values and corresponding osmolality using variables as yes/no, or hyperhydrated/not hyperhydrated.

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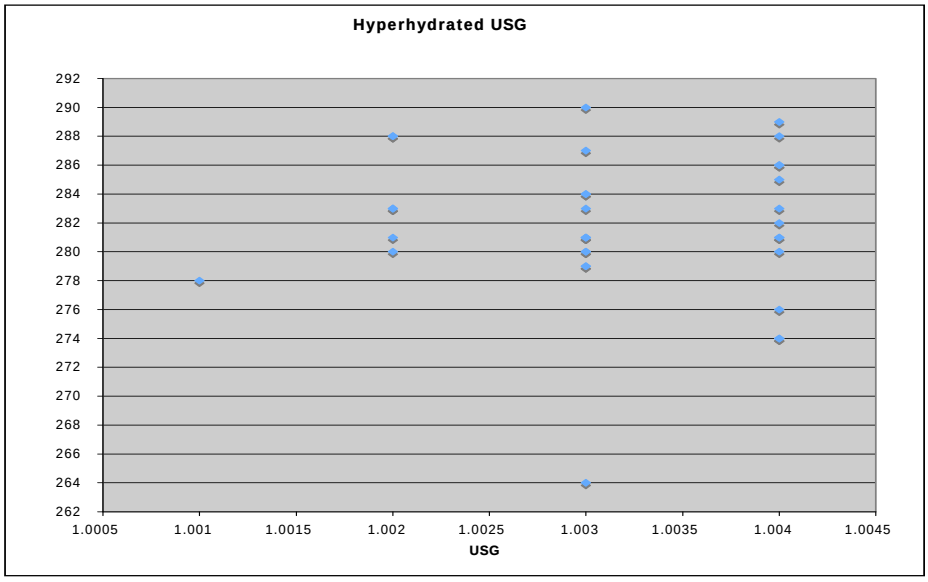

The final analysis to identify potential relationships was to transform both USG and osmometry to dichotomous variable and perform the Chi-square test. The threshold to define dehydration was 275 for osmometry and 1.003 for USG. Comparison using the Pearson Chi-square test resulted in p=0.507, also indicating no significant relationship. No significant relationships to determine hyperhydration were identified in the dataset using multiple methods of categorizing hyperhydration.

CHAPTER 6: DISCUSSION

USG via refractometry has been suggested to be an adequate indicator for hydration status when compared to blood osmolality.[4, 16, 28] Blood osmolality is typically used in clinical settings to ensure adequate hydration of blood samples. To limit the costs of evaluating potentially dehydrated blood, assessment of hydration status using refractometry demonstrated to be a specific non-invasive predictor of dehydration.

The mean USG of this free-living female runner sample was 1.008. According to any of the guidelines previously discussed, 1.008 falls in the well-hydrated category. The well-hydrated state is likely the outcome of being instructed to be attentive to fluids the night before and the morning of the visit. The urine samples collected were not initial morning samples, and the subjects were not strictly instructed to ingest a certain amount of fluid with very specific timing before visiting the laboratory (i.e. not enough control within the hour before the visit). Therefore, drinking fluids immediately before or at the lab visit would potentially impact both blood osmolality and USG values in an unpredictable fashion. Stover et al. controlled for this as they administered a survey to free-living exercisers to gather information concerning recent fluid intake and daily exercise regime.[28] Furthermore, in hopes to avoid the subjects from changing their fluid intake behavior, Stover et al. took the samples as soon as the subjects came to the fitness centers. Their study indicated a much higher correlation (r=0.99) between the urine and blood indices. Fluid behaviors immediately prior to these sorts of studies are likely critical to the reliability of the data.

The NCAA guidelines were implemented into this study to define hydration. The threshold defining dehydration was set at 1.020, with any USG value below or equal to 1.020 considered euhydration, and anything above 1.020 indicating hypohydration. 1.020 was set as a simple threshold without creating numerous ranges such as the NATA guidelines does. Using this threshold of 1.020 was consistent with the literature as it is commonly used as the cusp between euhydration and hypohydration among authors. This study was not designed to look at the range of hydration and dehydration values, and the sensitivity (false negatives) of the 1.020 USG could not be evaluated. The specificity turned out excellent as all of the blood results indicated that there was not one dehydrated sample in the group.

Other dehydration thresholds have been proposed and used. Armstrong et al. Stover et al. all defined the mean USG among their populations to establish a range for euhydration, using standard deviations below and above the mean to define hyperhydration, and hypohydration, respectively.[4, 28] Using this method with the current data did not yield any significant relationship; blood osmolality and USG did not correlate any when using the range of 1.003-1.013 as the range for euhydration. When looking at one standard deviation below this range, hyperhydrated USG values often demonstrated normally hydrated blood osmolality levels. This poor relationship is likely the outcome of the poor correlation of urine and blood values in our sample, and not necessarily reflective of poor threshold methodology. However, it must be noted that one cannot determine the mean value of a sample until the data has all been collected which would render this an unusable screening method unless the mean and distribution of another study were assumed as true.

Using 1.020 as a cutoff for hypohydration allowed our study to error on the side of caution. Since USG was used as pre-screening technique before blood was drawn, the USG value of 1.020 saved in terms of costs to evaluate blood osmolality. Stover et al. suggest that 1.025 can potentially be used as cutoff for recreational exercisers as they are not exposed to the same rigorous training of athletes.[28] It was their assumption that free-living exercisers would represent a more hydrated group compared to athletes.[28] However, the more stringent cutoff typically used for athletes was used even though we were studying recreational exercisers. In addition, since we used 1.020 as the threshold and did not take blood if the subject was considered hypohydrated, we were not able to evaluate how dehydrated USG values correlated with blood osmolality.

Popowski et al. defined euhydration in terms of blood osmolality as a range of 280-290osm/L.[22] Our study used a larger range of 275-295osm/kg to represent adequate hydration per clinical laboratory guidelines for the medical center. In general, using a range of 20 mosm with 295 as the top threshold (dehydration) and using refractometry as a pre-screening tool for USG values, all of the subjects fell in range of adequate hydration (no dehydrated subjects). Therefore, refractometry served its purpose in this study as it conveniently screened for dehydration as an indicator whether to proceed with the blood draw from the subjects. Ultimately, this proved to be cost effective for the lab as each blood draw costs approximately $150. There were 15 subjects who screened as being dehydrated using refractometry, which saved the laboratory a total of $2,250 in blood draw costs. Though we concluded the refractometry threshold for urine of 1.020 was adequate to prevent drawing and analysis of dehydrated blood, apriori we were not as concerned as we could have been about drawing

overly hydrated blood. More work is needed to define a reasonable threshold for overly hydrated.

From this study and the return of overly hydrated samples, it can be speculated that there is lag time of urine compared to blood.[22] Subjects were encouraged to be well hydrated and many took this comment seriously reporting to the lab with a water bottle in hand. Because fluid behaviors the morning of the study were not recorded, we cannot evaluate the impact immediate fluid consumption might have on the disconnect between blood and urine, but it is likely that the lag time between absorption of fluid into the blood and filtering of excess fluid via the kidney are responsible for the non-existent correlations. Even many of the hyperhydrated or very low USG values in this study, such as 1.003, fell within range of 275-295osm/L supporting that some of the subjects may have been within this lag period. Similarly, Popowski et al. attributed this lag time as a possible reason for the moderate correlation they found between USG and blood osmolality.[22] Research around the lag time is scant and this is an area for further research to help better design protocols that ensure hydration using the refractometer as a convenient predictor of hydration. It is possible in this study that limiting fluids in the hour immediately prior to the visit would have allowed the subjects time to reach fluid homeostasis and preserved the supposed relationship between blood osmolality and urine specific gravity.

The reason for evaluating hydration status of the blood is to avoid interpretation errors when looking at the clinical values of markers of interest in the blood. In looking for evidence of the female athlete triad, this study examined blood samples for high levels of cortisol and

thyroid stimulating hormone. Hyperhydration would dilute these values so the potential interpretation error is on the side of safety. A high cortisol (as reported in the study) may be even higher if the blood was euhydrated for this measure. The study also desired to look for low levels of free thyroid hormone, ferritin, vitamin D, and albumins. Hyperhydrated blood would potentially report these values as falsely depressed, and caution is warranted when interpreting these values for hyperhydrated subjects. Statistical manipulation of those values to adjust for hyperhydration may help in evaluation of the values.

CHAPTER 7: LIMITATIONS

The purpose of this study was to evaluate the utility of urine refractometry as a quick and non-invasive screening tool to ensure hydration status of serological measures. This study was not designed to evaluate the correlation of blood osmometry to urine specific gravity throughout the spectrum of hyper- to hypohydration. Additionally, the consumption of snacks and fluids in the time frame immediately preceding the measures was not tightly controlled or documented. The results of this study are limited to well-hydrated similar populations, and more importantly are subject to wide variation due to the lack of consistent and unknown pre-visit fluid consumption. Future considerations would measure the blood and serum throughout the spectrum of hydration and would follow a firm protocol for hydration prior to the visit so that the kidneys have ample time to clear extra fluids consumed. The hopeful outcome would be the ability to also predict hyperhydration using urine refractometry, and possibly determine a threshold of USG as a screening value for hyperhydration.

CONCLUSIONS

For this specific study, refractometry was a reliable method in terms of foreshadowing dehydration status before performing phlebotomy. The correlation between blood and urine values was not significant despite our previous prediction that USG and blood osmolality would be strongly correlated. However, certain limitations of this study may have significantly affected these conclusions. If we want to ensure that subjects are also not hyperhydrated, it is critically important to ask subjects of this study to follow a very specific hydration protocol that allows for enough time to clear the extra fluid from blood via the kidneys and bladder.

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