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Hydration biomarkers: Creating a user-friendly hydration assessment technique

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Hydration Biomarkers:

Creating a New Hydration Assessment Technique

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Chapter 1: Introduction

Water is the most important nutrient for the maintenance of life. It is essential for metabolism, temperature regulation, tissue structure and transportation of nutrients and waste (Raman et. al, 2004). Being at an optimal hydration level, termed "euhydration", is important for acute and chronic health. Acute hypohydration (i.e., lower than normal body water level) leads to temperature regulation impairment, increased cardiovascular stress, decreased exercise capacity and performance, impaired cognitive function, and diminished mood state (Armstrong, Costill, & Fink, 1985; Ganio et al., 2011; Hamilton, Gonzalez-Alonso, Montain, & Coyle, 1991; Montain & Coyle, 1992). Chronic hypohydration can lead to kidney stones, constipation, colorectal cancer, adenomatous polyps, and bladder cancer (Manz, 2007). Medical maladies linked to chronic hypohydration cost our healthcare system over $446 million a year (Warren et al., 1994).

Currently, suggested dietary recommendations exist to guide individuals with their fluid intake to maintain euhydration. Several fluid recommendations and hydration assessment techniques have been made in order to provide the public with easy guidelines to stay hydrated. Currently, popular techniques target physiological cues, dietary needs, and/or urine color analysis as hydration assessment techniques (Adolph, 1948; Armstrong, 2007; Armstrong et al., 1994). Some water intake guidelines instruct individuals to "drink to thirst" or drink 8 ounces of water 8 times a day (i.e. the "8x8 rule"; Valtin, 2002). Clinical guidelines suggest thirty-five milliliters per kilogram body mass of fluid should be ingested each day (Mudge & Weiner, 1990). The Panel on Dietary Reference Intakes for Electrolytes & Water, Standing Committee on the Scientific Evaluation on Dietary Reference Intakes suggests different water intakes for various ages and sexes (2.7 L for women, 3.7 L for men; Panel on Dietary Reference Intakes, 2004). However, sex, age, body compositions, activity level, environment, and health conditions

like illness and pregnancy all change the amount of water an individual needs to maintain euhydration (Latzka & Montain, 1999; Sawka, Cheuvront, & Carter, 2005; Manz, 2007).

No current recommendations take all physiological factors into account. For example, drinking to thirst cannot be trusted as an accurate guideline for individuals wanting to remain in a euhydrated state because the hypothalamus does not trigger thirst mechanisms until the body has lost 1-2% of body mass (i.e., 1-2% hypohydrated; Adolph, 1948). Therefore, individuals will already be hypohydrated by the time they feel thirsty. Setting a specific goal for all individual's water intake, like the 8x8 rule or 35 ml/kg body mass, is not ideal either because generalizing fluid intake to a set of "rules" for the entire population neglects several important factors such as body type and activity level. Even the guidelines suggesting different intakes for different ages or sexes do not account for physiological difference between body types, environmental conditions, or activity level. In summary, present guidelines and easy-to-remember axioms for fluid intake are not individualized, and thus do not accurately prescribe fluid intake for individuals of varying physiology.

Regardless of the fluid intake recommendation used, there needs to be accurate methods for assessing one's current hydration status. An optimal user-friendly technique should utilize little or no equipment and be an indication of total body water content, which is a dynamic measurement. Body water content naturally oscillates due to the dynamic fluctuation of water intake and water output. Current lab techniques for doing so can be accurate, but are costly, time consuming, and require technical expertise. This makes most laboratory methods impractical for the public's personal assessment (Armstrong, 2007). No one "gold standard" for total body water measurements is currently available (Armstrong, 2007). Various urine measures serve as great tools for hydration assessment since urine reflects net body water content (Sawka et al., 2005).

Armstrong, in 1994, introduced a user-friendly hydration assessment. He created an 8-level color scale to investigate whether urine could effectively analyze hydration level. Investigators correlated visual urine color assessment with laboratory measures of osmolality and urine specific gravity. Investigators concluded that urine color was an accurate measurement for hydration in a field setting (Armstrong et al., 1994).

Although urine color can be an accurate index for hydration analysis, it has shortcomings. Urine color analysis is unreasonable and uncomfortable for the public because accurate urine color cannot be determined by observing urine in a toilet. An accurate color can only be assessed from a sample provided in a clear container and assessed in a well-lit room (Armstrong et al., 1994). However, a study by Fletcher, Slaymaker, Bodenham, & Vucevic (1999) found a relationship between urine color and urine output, suggesting that urine output could accurately illustrate hydration level. He found significant correlations with urine output and urine color, specifically darker urine, known to be hypohydrated, correlated with less urine output volume.

Urine formation occurs in the bladder and when it reaches 40-50% capacity the pons, cingulated, frontal lobes, and peri-aqueductal grey regions of the brain activate and increase one's perceptual urge to void (Athwal et al., 2001). The urge to void corresponds with how much urine is in the bladder, and urine formation is correlated with total body water balance (i.e., more urine will form when there is a greater positive water balance; Athwal et al., 2001). In Athwal's study, subjects correlated a high urge to void when the bladder was filled with a higher volume, and correlated with a low urge to void with the bladder was empty or filled with low volumes. Trends in the study also illustrated the higher the volume, the more likely activation of bladder output will occur (Athwal et al., 2001). When making the assumption that increased urine output

directly correlates with number of voids, it may be possible to simply use the number of voids over a 24-hour time period as a user-friendly measure of hydration.

The primary purpose of this study is to use void number as a correlation to hydration status. We hypothesize that counting the number of urine voids over a 24-hr period will give the public an easy, equipment-free, method for hydration assessment.

Chapter 2: Literature Review

Current hydration measurements

Hydration measures assess the balance of water in the body, or net water balance. The body works under a variety of conditions, all of which have an optimal standard. Water content in the body is no different. The level of body fluid content can be described by three physiological terms: euhydration, hyperhydration, and hypohydration. Euhydration is defined as "optimal body water content," and is a sine wave around an average that is optimal for health and well-being (Armstrong, 2007). Hypohydration is uncompensated water loss via sweat, urine, feces, and respiratory vapor, reducing the total body water content (Institute of Medicine and Food and Nutrition Board, 2004; Oppliger & Bartok, 2005). Hyperhydration is a state when fluid intake is greater than optimal and the total body water content rises above the average point (Armstrong, 2007).

Fluid guidelines

The 8 x 8 rule (8 glasses of 8 ounces a day) is often accepted as a proper hydration guideline. However, there fails to be scientific evidence supporting this as a proper hydration technique (Valtin, 2002). Age, sex, environment, activity levels, and diets constantly change, making a definite water intake amount an unfeasible calculation for everyday hydration. Another guideline professionals suggest to the public is to "drink to thirst." However, drinking to thirst is not ideal for euhydration. The hypothalamus does not arouse thirst perception until the body has lost 1-2% of body mass, according to Adolph's study in 1948. At this point, the body is already in a hypohydrated state (Adolph, 1948).

Current guidelines fail to account for the differences in total body water turnover between ages and sexes (Sawka et al., 2005). Fluid balance studies show that average water requirements increase from infancy to adulthood (Table 1). Infants require about 0.6 L a day, children require about 1.7 L, while adults require above 2.2 L (Sawka et al., 2005). The Journal of American College of Nutrition suggests recommendations for different ages and sexes, but still fails to account for activity and varying physiologies (Panel on Dietary Reference Intakes, 2004).

Table 1. Total water recommendations for age and gender.

Life stage group	Total water L/day
Infants 0-6 months	0.7
Infants 7-12 months	0.8
Children 1-3 years	1.3
Children 4-8 years	1.7
Boys 9-13 years	2.4
Girls 9-13 years	2.1
Boys 14-18 years	3.3
Girls 14-18 years	2.3
Men 19-70+	3.7
Women 19-70+	2.7

Advanced age has not thoroughly been studied, but a decline in physical activity and fluid regulatory capacity due to declining renal concentrating and diluting capacity could alter the hydration requirements for elderly individuals. Sex also affects the daily fluid requirements because women exhibit significantly lower water turnover rates than men (Sawka et al., 2005). Health conditions also affect the turnover in individuals. Illnesses, pregnancy and breastfeeding can cause fluid turnover changes (Manz, 2007). Some illnesses cause hypohydration while other cause hyperhydration. It is clear that making fluid recommendations to fit everyone is impossible (Manz, 2007). Independent of age, sex, and health status one of the biggest factors effecting

daily water turnover is activity level. For example, a sedentary man needing a daily water intake of 2.5 L a day may need as much as 3.2 L a day if large sweat losses occur during physical activity. The environment plays a role in increasing hydration needs; if the same male is exercising in the heat, he may need 6 L of water per day (Sawka et al., 2005).

Nearly all guidelines are measured as one specific point, but body water content is not a static measure. Body water content oscillates due to the dynamic fluctuation of water intake and water output. Water intake is calculated by food and fluid intake. Sweat, urine, feces, metabolism, and respiratory vapor contribute to water output (Armstrong & Casa, 2009). A new hydration guideline should utilize body functions instead of behavioral functions or blanket guidelines for everyone as an indicator of hydration status. The bodily function most closely representing net water balance is urine output (Sawka et al., 2005). Urine output directly reflects net fluid levels. A new guideline should utilize urine output measurements as a hydration assessment since urine output most closely reflects net body water level.

In sedentary individuals ~5% to 10% of total body water is turned over daily due to basal functions (Sawka et al., 2005). Urine output produces about 1 to 2 L of water turnover daily, but that number changes dramatically depending on hydration status. Although water is lost through other bodily functions, at rest, urine is the primary avenue for water losses. Urine void fluctuates according to net water balance, indicating urine is the primary avenue to regulate net body water balance across varying intake volumes (Sawka et al., 2005). Urine indices are based on the relationship between arginine vasopressin (AVP) and urine osmolality and urine specific gravity (USG). The AVP system and renin-angiotensin system primarily control renal functions. AVP is the main regulating hormone for body fluid (Norak, 1996). Plasma volume regulates osmotic pressure changes (Norak, 1996), which in turn stimulate the AVP levels; small levels of pressure

increase AVP levels (Birnbaumer, 2000; Ritz, 2001; Sawka et al., 2005). Urine formation occurs in the bladder and when it reaches 40-50% capacity the pons, cingulated, frontal lobes, and peri-aqueductal grey regions of the brain activate and increase one's perceptual urge to void (Athwal et al., 2001). The urge to void corresponds with how much urine is in the bladder, and urine formation is correlated with total body water balance (i.e., more urine will form when there is a greater positive water balance) (Athwal et al., 2001). In Athwal's study, subjects correlated a high urge to void when the bladder was filled with a higher volume, and correlated with a low urge to void with the bladder was empty or filled with low volumes. Trends in the study also illustrated the higher the volume the more likely activation of bladder output will occur (Athwal et al., 2001). When making the assumption that increased urine output directly correlates with number of voids, it may be possible to simply use the number of voids over a 24-hour time period as a user-friendly measure of hydration.

This is relevant because greater water intake suggests a greater water amount filters through the renal system instead of going through absorption. The amount of water present in the filtered urine of the bladder will determine the concentration of urine and distinguish hydration status in individuals. However, there is one setback to urine output measurements. The volume and timing of voids is important to the individual's hydration. Taking a large volume of water in a short amount of time stresses the renal system. The system registers the water as harmful to the body. Therefore, the renal system filters much of the water, not absorbing, even if the body is in a hypohydrated state. Therefore, one urine measurement is not reliable as a sole measurement of hydration status (Armstrong et al., 1994). A 24-hr period of urine needs to be collected for a more representative indication of hydration status.

Current lab assessments and techniques for measuring hydration status

Current lab assessments fail to create a user-friendly device or assessment for everyday public use. The current assessments are either impractical or unreliable. Many experiments require time and money that the general public does not have (Armstrong, 2007). The current claim is that measuring total body water (TBW), or all the fluid in and out of the cells, along with plasma osmolality, are "gold standards" for hydration assessment (Armstrong, 2007). The most accepted methods of TBW measurements are the stable isotope dilution and neutron activation analysis techniques (Fletcher et al., 1999; Institute of Medicine and Food and Nutrition Board, 2004; Ritz, 2001; Sawka et al., 2005). However, body water content is continuously changing (Institute of Medicine and Food and Nutrition Board, 2004), and the current "gold standard" is not able to detect changes in TBW throughout the day. Although stable isotopic dilution is one of the most accepted measures, it is taken once and delivers one set point of hydration. In addition, stable isotopic dilution is virtually unattainable for the general public. The general public has limited, if any, access to stable isotopic dilution due to high costs, low portability, and required technical expertise. Neutron activation analysis includes many of the same problems. Neutron activation analysis is a technique used to find radionucleotides by radiating a sample and analyzing the gamma ray decay (Armstrong, 2005). Neutron activation analysis requires high technical expertise. The equipment for this assessment is costly and has low portability. Assessment run time is lengthy and the likelihood of an adverse event is moderate based on Armstrong's hydration assessment method analysis (Armstrong, 2007). In addition, these tests not being portable, provides even greater difficulty for the general public to follow through with continuous testing, and thus the public is left without a proper assessment tool. Various techniques and their shortcomings and benefits can be seen in Table 2.

Table 2. Selected Characteristics of 14 Hydration Assessment Methods (Armstrong, 2007).

Hydration Assessment Technique	Body Fluids Involved	Cost of Analysis	Time Required	Technical Expertise Required	Portability	Likelihood of Adverse Event
Stable isotope dilution	all (ECF and ICF)	3	3	3	3	2 or 3
Neutron activation analysis	all	3	3	3	3	2
Bioelectrical impedance spectroscopy (BIS)	uncertain	2	3	2	2	1
Body mass change	all	1	3	1	1	1
Plamsa osmolality	ECF	3	1	3	3	2
% plasma osmolality	blood	2	2	3	3	2
Urine osmolaity	excreted urine	3	2	3	3	1
Urine specific gravity	excreted urine	1	2	2	1	1
Urine conductivity	excreted urine	2	1	2	3	1
Urine color	excreted urine	1	2	1	1	1
24-hour urine volume	excreted urine	1	1	1	1	1
Salivary flow rate, osmolality, total protein	whole, mixed salvia	2 to 3	1	3	2 to 3	1
Rating of thirst	hypothalamus	1	2	1	1	1

Key to ratings	1 = small
	2 = moderate
	3 = great

Despite difficulty in a laboratory setting, plasma osmolality, stable isotope dilution, urine osmolality and urine osmolality provide valid assessments of hydration status (Armstrong, 2007; Fletcher et al., 1999). For example, population data suggest that individuals with a 24-hour urine volume of approximately 1.4 L, a 24-hour urine specific gravity ≤ 1.020, and/or a 24-hour urine osmolality ≤ 766 mOsm/kg are euhydrated for males (Table 3; Armstrong et al., 2007); female values are similar but not the same. (Table 4; Armstrong et. al., 2012).

Table 3. Hydration values for a 75-kg male (Armstrong et al., 2007).

Hydration category	Percentile range (percent)	Morning serum osmolality (mOsm/kg)	24-hr total fluid intake (mL)	Morning U_{SG}	24-hr U_{SG}	Morning U_{Osm} (mOsm/kg)	24-hr U_{Osm} (mOsm/kg)	Morning U_{Col}	24-hr U_{Col}
Slightly hyperhydrated	11-25	285-28	1,898-2,250	1.017-1.021	1.012-1.014	545-713	377-475	4	4
Well hydrated	26-40	287-288	1,526-2,897	1.022-1.023	1.015-1.017	714-817	476-586	5	5
Euhydrated	41-60	289-291	1,226-1,525	1.024-1.026	1.018-1.020	818-924	587-766	5	5
Slightly dehydrated	61-75	292	1,075-1,225	1.027-1.028	1.021-1.024	925-999	767-880	5	6
Very dehydrated	76-90	293-296	875-1,074	1.029-1.031	1.025-1.027	1,000-1,129	881-1,013	6	6
Extremely dehydrated	91-100	>296	<875	>1.031	>1.027	>1,129	>1,013	>6	>6
95% confidence interval	2.5-97.5	274-301	675-3,000	1.011-1.033	1.009-1.030	377-1,194	281-1,114	3-7	2-7

Table 4. Hydration values for a 59.6 kg female (Armstrong, et al., 2012).

Hydration category	Percentile range (percentile)	Total fluid intake (mL)	Beverage intake (mL)	Urine volume (mL)	USG	24-U_{Osm} (mOsm/kg)	24-hr U_{Col}
Increasing hyperhydration	1-10	>3,407	>2,804	>2,070	<1.008	<320	<3
	11-25	2,946-3,407	2,471-2,804	1,828-2,070	1.008-1.011	320-382	3
	26-40	2,507-2,945	1,832-2,070	1,240-1,827	1.012-1.015	383-548	4
Euhydrated	41-60	2,109-2,506	1,831-1,154	1,240-1,827	1.012-1.015	383-548	4
	61-75	1,745-2,108	1,154-1,299	831-950	1.021-1.023	706-809	5
Decreasing dehydration	76-90	1,507-1,744	954-1,153	531-830	1.024-1.026	810-863	6
	91-100	<1,507	<954	<531	>1.026	>863	>6
95% confidence intervals	2.5-97.5	658-4,152	135-4,152	5-2,599	1.004-1.030	118-1,091	2-7

Although appropriate in laboratory settings, the expense, time, convenience, and expertise needed to obtain these measures preclude them from being used on a daily basis by the general population. However, in 1994, Armstrong et al tried to remedy the impracticality of hydration assessments and developed a urine color chart. The urine chart utilizes degrees to which urine is concentrated with a yellow color corresponding to one's hydration status, "pale yellow" and "straw color" are examples of euhydration (Armstrong et al., 1994). Although the urine color chart eliminates expense and complicated technical expertise, there are still inconveniences to the public. For highest accuracy, the sample must be placed in a clear container and visually analyzed in a well-lit room to a standardized urine color scale; examining color in a toilet is invalid. The specifics of analyzing urine color can be uncomfortable and unattainable, inconveniencing the general public. In addition, certain vitamins and medications can alter urine color (Panel on Dietary Reference Intakes, 2004).

Creating a new, user-friendly hydration technique

A new user-friendly hydration technique requires several criteria. First, the technique should be accurate. Accuracy for urine assessment will depend on whether the measurement can track the oscillating measure of body water content. Usability for the general public is another goal of creating a new user-friendly technique. The elimination of technical equipment or equipment all together, will increase the usability of a hydration technique by the general public. To increase practicality the assessment should be portable or easy to track. Tracking urine output would fulfill these requirements. Urine output reflects net body water, meaning it would be an oscillating measure (Sawka et al., 2005). Counting urine void number and having a correlated number reflecting hydration status would be a tool requiring no equipment or expertise. This experiment will test the validity of measuring urine void number as a marker hydration status.

Chapter 3: Materials and Methods

Subjects

Subjects (n = 47) tested were between 18 to 39 years old and were healthy males and females from the University of Arkansas and surrounding area. The subjects were required to fill out a medical questionnaire and only those not suffering from metabolic, neurological or cardiovascular illness participated. Specifically, no individuals on medications affecting kidney function participated, including individuals taking anti-hypertensive medications. Individuals were weight stable and were not pregnant, as this affects the physiological systems investigated. An institutionally-approved informed consent was signed by participating individuals.

Experimental Trial

Subjects met in the Human Performance Laboratory in the HPER Building at the University of Arkansas. Upon signing the consent form, the participant's age, height, and body mass were measured. Body mass was recorded to the closest 100 g using a scale (Seca, model: 7701321004, Vogel & Hamburg, Germany or Health-o-meter), and standing height was measured with a wall-mounted stadiometer (Seca, model: 770, Vogel & Hamburg, Germany or Seca "Accu-Hite"). Body weight and height were recorded with the subjects wearing minimal clothing and with no shoes. Body composition (% fat) was measured with the same hand-to-foot bioelectrecal impedance analyzer for each participant (BIA; Quantum - RJL, MI, USA) at a fixed signal frequency.

Subjects were provided fluid and food logs and were instructed on the proper use of them. Subjects were asked to refrain from alcohol and caffeine during the observational period since their effects on hydration are not fully understood. In addition, the subjects were not told

the specific hypothesis of the project in order to keep their daily habits as similar to normal routine as possible.

Participants received one large urine collection container and two small urine collection containers. The subject used the large container for collecting urine over a 24-hour time period. The two smaller containers were used as part of a larger research study. The subjects provided two separate forced samples in the smaller containers: a single forced lunch void and a single forced morning void. Each container was labeled with collection type (i.e. lunch, morning, 24-hour), date of collection, and simple reminders for each void data recordings. Subjects were instructed to void at "normal desire" throughout the course of 24-hr urine collection, and were educated on the difference between first, normal, and strong desire to urge (Athwal et al., 2001). For each void provided in the 24-hour container, participants used the 0-4 perceptual urgency scale and marked their corresponding score. The participants were instructed that $0 = $ no sensation; $1 = $ first sensation; $2 = $ first urge to void; $3 = $ strong urge; $4 = $ uncomfortable urge. Subjects were asked to void at a 2, or first urge to void, on this scale. In addition, after each void, the subject marked the level of urine along with the time of day and their urge to void score on the side of the container with a provided pen. This provided investigators with the frequency of void throughout the 24-hour time period. The container had instructions reminding the participant of the void recording requirements, the urge to void scale and a thirst scale (recorded after each void).

After obtaining research materials, the subjects started the study the next morning. The first day of participation required the subject to only record all fluid, food, medications, and supplements. This allowed for a stabilization period before the 24-hr urine collection day. The second day of participation the subject continued to record fluid, food, medications, and

supplements in addition to collecting the 24-hour urine sample and the forced lunch void. However, the subject was instructed to void their first void of the day into the toilet, starting the 24-hour collection the void following the first morning void. The 24-hour collection ended on the morning sample after the day of urine collection. Thus, the forced morning void was collected upon waking on the third day of participation. If the subject awaked during the night with an urge to void, the subject was instructed to void into the 24-hour container. Any voids prior to 5AM or prior to the subject waking the next day were collected in the 24-hour container.

    As soon as possible, after the morning void collection on the third day of participation, the subject reported back to the laboratory where they returned urine collection containers and fluid and diet logs. A follow-up meeting insured they complied with pre-test instructions, specifically whether or not they followed instructions to void at a "normal desire." Volume of each smaller container was measured using a balance beam, tared using a replica of the container. Then the volume for the 24-hour container was measured with the morning, lunch, and 24-hr sample, again tarred using a replica of the container. Lunch and morning urine samples were measured first, and then the two smaller, forced samples were mixed into the 24-hour container for a true 24-hour sample. After finding the total volume number, separate void numbers were estimated by refilling the container with fluid to the estimated participant markings and measuring the fluid level, after taring the container. Hydration status of the 24-hour, lunch, and morning samples were assessed by analyzing a well-mixed sample of each collected sample mixed as one, using various markers of hydration status as performed by others (Armstrong et al., 1994; Armstrong et.al., 2005). Specifically, urine color, urine specific gravity (USG), and urine osmolality (U_{OSM}) were assessed using standard hydration techniques (Armstrong, 2007; Armstrong et al., 1994). The Armstrong Urine Color Scale assessed each urine sample color and was measured by the

same researcher in the same well-lit environment throughout the duration of the study (Armstrong, 2005). An ATAGO SUR-NE refractometer assessed urine specific gravity. Lastly, urine osmolality was assessed with freezing point depression (3D3 Advanced Instruments osmometer). Void volume was estimated by comparing each demarcation made by participants to a standard, pre-marked container. The number of voids was counted from the number of demarcations indicated.

Data Analysis. Subjects were grouped by level of hydration as defined by Armstrong et al. (2005; 2012). A USG $\leq$ 1.021 was considered euhydrated; USG > 1.021 were considered hypohydrated (Armstrong, 2007; Armstrong 2012). $U_{OSM} \leq$ 728 mOsm/kg H_2O was classified as euhydrated; U_{OSM} > 728 mOsm was classified as hypohydrated when examining male and female data together (Armstrong, 2007; Armstrong 2012). Females with $U_{OSM} \leq$ 705 mOsm were considered euhydrated, and males with $U_{OSM} \leq$ 766 were considered euhydrated (Armstrong, 2012).

Data was analyzed using Pearson-product correlations to evaluate the relationship between number of voids and various hydration indicators (urine specific gravity and urine osmolality). Statistical t-tests were used to compare euhydrated and hypohydrated groups using urine specific gravity and urine osmolality. A two-way analysis of variance (ANOVA) was used to evaluate if the number of voids between different levels of hydration differed because of sex (i.e., statistical interaction). Significance was determined by having an alpha level of 0.05.

Chapter 4: Results

Level of urge upon voiding was consistent throughout the study (2 ± 0), suggesting participants complied with protocol. As expected, using established cutoffs resulted in significant differences between euhydrated and hypohydrated assessments for 24-hr urine specific gravity, 24-hr urine osmolality, 24-hr urine color, and 24-hr urine volume (Table 5, $p < 0.01$). When combining the data from both the men and women, there was a significant difference in void numbers between euhydrated (n = 43) and hypohydrated (n = 6) individuals (Table 5, $p < 0.05$). A moderate correlation was observed between urine osmolality and void number ($r = -0.38$, $p < 0.05$). Correlating number of voids to urine specific gravity yielded similar results ($r = -0.37$, $p < 0.05$). Individual voids were 264 ± 103, 296 ± 97, and 233 ± 101 ml for the total, male, and female groups, respectively.

After analyzing the overall population, sex-specific analyses were performed. As expected, the hypohydrated (n = 6) males had a higher 24-hr urine color, urine specific gravity, urine osmolality, and lower urine volume, compared to the euhydrated males (Table 5, $p < 0.05$; n = 19). Void number was not significant when comparing male hypohydrated sample with the male euhydrated sample (Table 5).

Females followed the same trend. Significant findings were evident when comparing euhydrated females (n = 22) to hypohydrated females (n = 2) in urine specific gravity, urine osmolality, urine color, and urine volume over the 24-hr time period (Table 5, $p < 0.05$). However, void number was not significantly different when comparing female hypohydrated sample with the female euhydrated sample (Table 5).

When using osmolality to identify hydration levels, sample sizes slightly changed, but significance testing yielded the same results as when using urine specific gravity. The total

sample size still indicated all the hydration biomarkers tested, including number of voids, were significantly different between euhydrated and hypohydrated individuals (Table 5, $p < 0.05$). Using osmolality to define hydration state, there were 21 euhydrated and 3 hypohydrated females. Significant findings were still observed in urine specific gravity, osmolality, volume, and color for the 24-hr collection (Table 5, $p < 0.05$), but not in the number of voids between women (Table 5). In males the euhydrated (n = 20), still had significant findings compared to the hypohydrated males (n = 3) in urine specific gravity, urine osmolality, urine color, and urine volume for the 24-hr period (Table 5, $p < 0.05$), but not for number of voids (Table 5). An overview of voids between euhydrated and hypohydrated individuals can be seen between the overall, male, and female samples in Figure 1. There was not an interaction effect when analyzing sex and hydration together indicating that differences in void number where not dependent on sex.

   Number of voids was then correlated to urine osmolality (Figure 2) and urine specific gravity (Figure 3). As predicted, the number of voids compared to urine osmolality and urine specific gravity had an inverse relationship. A higher number of voids throughout a 24-hr period correlated with a lower urine osmolality and lower urine specific gravity; this was moderately and negatively correlated (USG: $r = -0.37$; U_{Osm}: $r = -0.38$).

Table 5. Mean ± SD 24-hr hydration markers for overall (n = 47), males (n = 23), and female (n = 24).

	Overall		Male		Female	
	Euhydrated	**Hypohydrated**	**Euhydrated**	**Hypohydrated**	**Euhydrated**	**Hypohydrated**
N	41	6	19	4	22	2
USG	1.012 ± 0.004**	1.023 ± 0.003	1.012 ± 0.004**	1.025 ± 0.004	1.012 ± 0.004**	1.028 ± 0.000
Color	2 ± 1**	4 ± 1	2 ± 1*	5 ± 1	2 ± 1**	5 ± 1
mOsm (mOsm/kg H$_2$O)	431 ± 143**	841 ± 137	456 ± 159**	879 ± 137	409 ± 147**	1020 ± 15
Volume (ml)	2007 ± 863**	1193 ± 434	2334 ± 1007*	1181 ± 369	1726 ± 569*	753 ± 251
Number of voids	6 ± 2*	4 ± 1	6 ± 2	4 ± 1	6 ± 2	4 ± 3

*Significantly different than hypohydrated at $p < 0.05$
**Significantly different than hypohydrated at $p < 0.01$

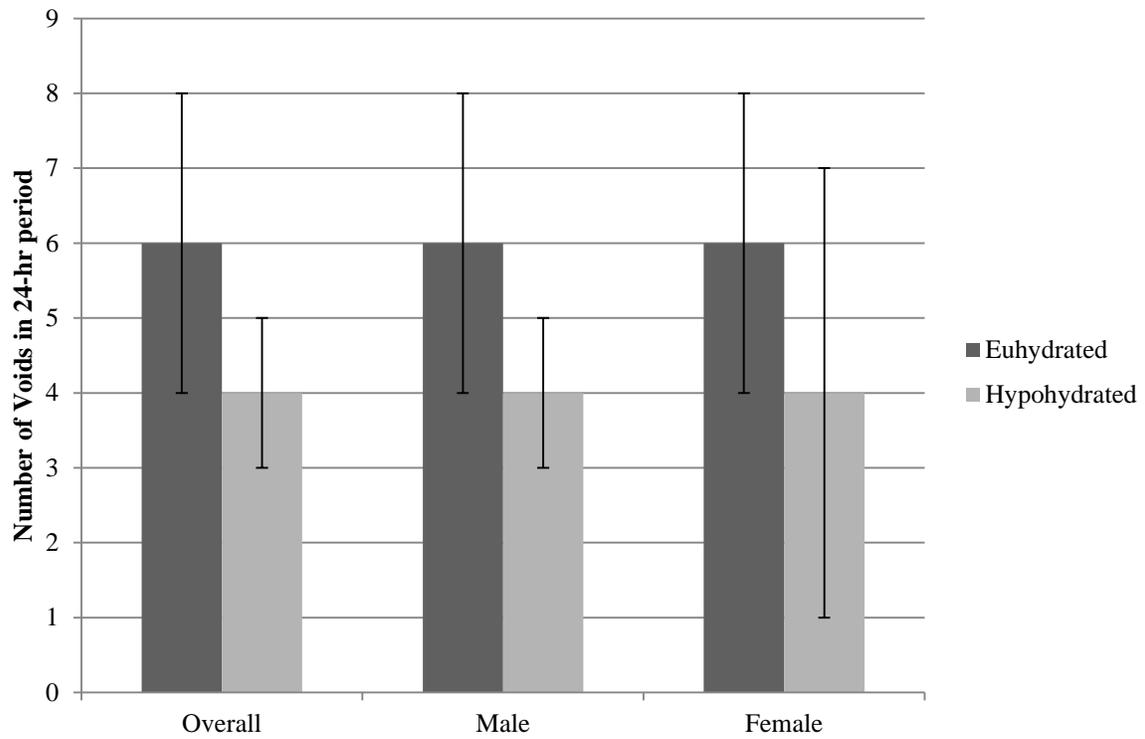**Figure 1.** Void numbers between euhydrated and hypohydrated overall, male, and female samples.

Figure 2. Void number correlated with urine osmolality, a currently well accepted hydration assessment technique ($r = -0.38$, $p < 0.05$).
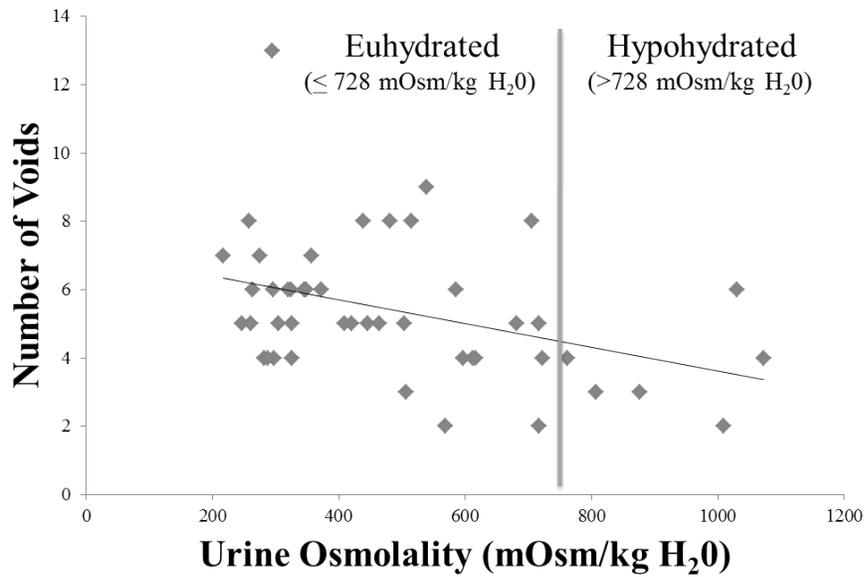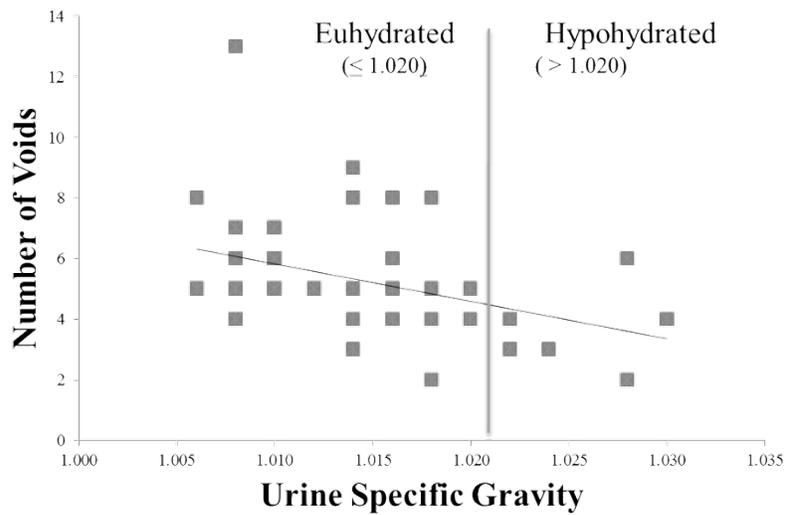

Figure 3. Void number correlated with USG, another currently accepted hydration assessment measure ($r = -0.37$, $p < 0.05$).

Chapter 5: Discussion

After having subjects collect urine for 24-hr in a free-living environment, we assessed their hydration status using standard techniques (USG and urine U_{Osm}). Throughout the 24-hour collection period the subjects marked each void on the container. This allowed us to accurately know how many voids occurred in the 24-hour period. After identifying euhydrated and hypohydrated samples based off of USG and urine osmolality, we were able to identify if the number of voids differed between euhydrated and hypohydrated subjects. When comparing the overall euhydrated sample to the overall hypohydrated sample, voiding 6 times throughout the day corresponded to being euhydrated, whereas voiding 4 times throughout the day corresponded to hypohydration. These results were the same for males and females; voiding 6 times throughout the 24-hr period was seen in the euhydrated male and female group, and 4 times throughout the 24-hr period was seen in the hypohydrated male and female group. Both males and females having the same voids corresponds to the insignificant differences seen in the interaction effect, illustrated hydration status did not depend on sex. Although number of voids for euhydrated and hypohydrated groups was the same between overall, male, and female poulations, when examining within-sex differences, the results were not significant. Void number was not significantly different between euhydrated and hypohydrated males or euhydrated and hypohydrated females. This was probably due to the small hypohydrated sample sizes in each of the male and female groups.

Importantly, subjects were asked to void each time they had a normal urge throughout the 24-hr period. This was an important control in the experiment because inconsistently voiding at different urges would have varied the number of voids. We carefully controlled for this variable by instructing individuals to comply with the urge 2 on the urge scale (Athwal et al., 2001).

Controlling for void urge allowed for a consistent void volume levels throughout the day. Athwal et al. (2001), illustrated urge to void corresponds with greater fluid volume in the bladder. Therefore, by controlling urge level, individuals were voiding similar volumes more often, rather than holding fluid, which might change void volume numbers. In addition, after placing samples in hydration categories, individuals who voided 6 ± 2 voids throughout the 24-hr period met the criteria to previous, validated research assessment techniques of urine specific gravity, urine osmolality, urine color, and urine volume for euhydration status. Simply, individuals having 6 ± 2 voids throughout the 24-hr period were euhydrated on all levels of known euhydrated measurements known in the literature (Table 5; Armstrong, 2007; Armstrong, 2012). Likewise, hypohydrated individuals revealed hypohydrated measures for urine specific gravity, urine osmolality, and urine volume (Table 5; Armstrong, 2007; Armstrong, 2012).

We observed individuals in a free-living environment. No special instructions were provided to subjects regarding how much fluid to drink. In our sample size, the majority of subjects were euhydrated. We had only 6 subjects that were identified as hypohydrated versus 41 euhydrated individuals. It is possible that knowing the study measured hydration status, individuals changed daily habits to increase fluid intake, and thus increase hydration levels. Thus, with unequal sample sizes our findings are statistically limited. Future studies should systematically hypohydrate individuals to examine the influence of hypohydration on void number.

Future research

To generalize the void number assessment to the public, future research should look at the effects of caffeine and alcohol on void number, because the consumption of these drugs occur in a large proportion of the population. Specific values between sex differences should be evaluated, and using a greater hypohydrated sample size will benefit future studies. Also, the

effects of maturation and aging should be investigated (children and elderly). In addition, medications or illnesses might have an effect on void frequency and should be examined further.

Conclusion

Ultimately, when comparing the euhydrated and hypohydrated individuals in the overall population using number of voids in 24-hours, it was easy, inexpensive, and comfortable to use for the public to self-assess hydration status. However, probably due to a small sample size, the same findings cannot be extended into specific information for males and females independently, and further research should increase hypohydration samples or control for hydration status by regulating fluid intake to determine more accurate values between sexes.

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