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*“Hydration Biomarkers and Water Intake in 3-13 year-old boys”*

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## ABSTRACT

**Background/Introduction:** Hydration is an important aspect of health in children. Despite being important, limited information is available regarding children's water intake and hydration.

Interestingly, most studies assess hydration status based on a single random urine sample instead of a 24-h one. **Purpose:** The aim of this study was to examine the prevalence of dehydration and water intake in boys 3 to 13 years-old.

**Methods:** Forty-six healthy boys were recruited for the study. Each subject recorded food and fluid intake in a diary on a Saturday and Sunday. They also collected their 24-h urine on Sunday. The 24-h sample was analyzed for urine volume,

osmolality (freezing point depression), color (8 color scale) and urine specific gravity (refractometry). The nutrition data system for research software was used to calculate water

intake from food from diaries. Water intake data were presented as the average of Saturday and Sunday. Physical activity was estimated via the international physical activity questionnaire and

data are presented as total MET-min per week. Mean values between groups were compared by student's t-test. Data were analyzed with JMP statistical analysis software. **Results:** Eighteen of

the 46 boys (39%) were dehydrated (urine osmolality  $>800$  mOsm/kg) and 29 of them (63%) did not meet the Institute of Medicine's dietary guidelines for daily water intake. The 24-h urine

osmolality for euhydrated and dehydrated boys was  $530 \pm 150$  and  $967 \pm 140$  mOsm/kg ( $P < 0.05$ ), respectively. Physical activity was not different between dehydrated ( $5,506 \pm 4,941$  MET-min/wk)

and euhydrated boys ( $4,575 \pm 4,338$  MET-min/week;  $P > 0.05$ ). Dehydrated boys had lower total water intake ( $1,661 \pm 759$  mL) than euhydrated ones ( $1,937 \pm 1,661$  mL). **Conclusion:** These

preliminary data suggests that the majority of 3-13 year-old boys fail to meet the water intake recommendations and 4 out of 10 are hypohydrated. More data are needed to examine the factors

that influence these observations.

## **Introduction**

Hydration is an important aspect of health. Water, often neglected as a macronutrient, is necessary for all individuals – young and old. Water is essential for our physiology. It is necessary for proper metabolism, thermoregulation and transportation of nutrients as well as waste products. Water is also key as a building material which means that it is of higher necessity during periods of time when the human body is growing. Chronic diseases have also been linked to poor daily hydration levels and water intake (Jequier & Constant, 2010). Water is essential to the health of the human cell. It is needed for physical performance and overall energy from day to day (Popkin, D’Anci, & Rosenberg, 2010).

While water and beverage consumption is the main contributor towards hydration state, food also contributes to overall water levels in people. There is a significant amount of research on adults and their hydration and water intake. Few research studies have been conducted to examine and understand general hydration status in children. Research completed in this area has shown that the majority of children are not at adequate hydration levels and that there are disparities between genders and ethnicities on levels of hydration (Kenney, 2015). Along with that, the Dortmund Nutritional and Anthropometric Longitudinally Designed Study concluded that the children who had a better or more preferred diet also had a higher hydration status (Stahl, 2007). Inadequate fluid consumption is correlated to dehydration which has significant negative side effects. The negative health implications of inadequate fluid consumption are physiological and cognitive. There is substantial research done on the negative effects of dehydration on metabolism, gastrointestinal function, kidney function, and cardiovascular function (Popkin, D’Anci, & Rosenberg, 2010). Ability to complete cognitive tasks have been found to be affected by lack of adequate fluid consumption in children (Edmonds, 2009). Because cognitive function

might be correlated with hydration status, it might be important for children to maintain hydration status while in school.. Even though it is widely known that water is a necessary aspect of daily living, most people do not drink adequate amounts. Consequently, children, who are mostly dependent on parental help, often don't drink adequate water either. In one study, it found that 75% of the children that participated in their study did not drink any water in the morning before attending school (Stookey, 2012).

Typically research on water intake has been focused on avoiding the negative effects of dehydration. Research is now starting to ask questions on how increased water intake could positively affect daily functioning. Research has overall been focused on the effects of hydration or lack thereof and so some reference measures have not been acquired. Inadequate hydration has been defined as having a urine osmolality equal to or greater than 800 milliosmoles per kilogram (Y-BAR, Urkin, & Kozminsky, 2005). Many measurements of hydration status exist, including measuring changes in body weight, tracer techniques, bioelectrical impedance, plasma osmolality and urine measurements. Urine measurements include urine osmolality, urine specific gravity (USG), urine color and 24-hour urine volume. (Jequier & Constant, 2010). Many studies have looked at the validity of these measurements and the best predictor of overall fluid intake (Armstrong, 2013, Kavouras, 2002, Perrier, 2012). Armstrong conducted a study that evaluated 12 different hydration biomarkers in order to which was the best at representing 24-hour whole-body water balance. This study concluded that the only hydration measure that predicted water balance was 24-hour urine osmolality and 24-hour urine specific gravity. Perrier had similar findings in which 24-hour urine osmolality and 24-hour USG along with urine color were strongly associated with total fluid intake volume. 24-hour urine samples are the most commonly used measurement of hydration status, along with body weight (Kavouras, 2002). Knowing that

this is considered the most sensitive measurement, there have still been no studies done in which 24-hour urine samples have been collected from children to predict water-intake for the general population.

Water intake and hydration status is an understudied topic, especially in children. Along with that, most studies thus far have not looked at aspects of hydration in children nationally. The National Health and Nutrition Examination Survey (NHANES) conducted a large study looking at different aspects of water consumption and inadequate fluid intake using extensive surveys and 24-hour recalls on fluid and food diaries and a single urine sample. This was the first study to look at children's general hydration status in the United States. This study found that more boys of the ages 6-19 had significantly higher urine osmolality's, meaning that they had higher concentrations of solute in the urine, compared to girls. They also found differences between ethnicities, and age in regards to hydration status (Kenney, 2015). They found that there was not a single group of children that came close to Dietary Reference Intakes for water, where the most successful group was 25% of 4-8yr olds who met the DRI's for water (Drewnowski, 2013). This study has a few major limitations. This study relied on subjects to recall what they had had to eat and drink on the previous day. It also only collected a spot urine sample, which as stated previously – isn't considered a comprehensive measurement of overall hydration status. Given that this was the first study conducted to look at national hydration status' in kids, there is still much that is unknown about children's hydration.

The Hydration Biomarkers in Kids Urine Samples (HYBISKUS) study will focus on establishing a reference for hydration biomarkers in boys ages 3-13 using 24-hour urine samples. We are focusing on developing foundational hydration biomarkers for future research nationally. With this data, we would expect that hydration status should be predictable in the subjects based

on these hydration biomarkers and total water intake. We examined 24-hour urine osmolality, urine specific gravity and color in male participants aged 3-13 years-old. We collected 48-hour fluid diaries and food logs to see if water intake is correlated with the hydration indices. The aim of this preliminary study was: to examine the prevalence of dehydration and water intake in boys of 3 to 13 years-old A secondary aim was to examine if boys were meeting the Institute of Medicine’s guidelines for total water intake, as well as collecting physical activity data to determine if physical activity effected hydration status.

### Methods

**Subjects.** Forty-six apparently healthy boys from 3 to 13 years-old were recruited into the study. The descriptive characteristics of the participants recruited is presented in the table below:

**Table 1:** Subject Characteristics: weight, height and BMI are presented as mean  $\pm$  standard deviation. Ages were put in three groups based on the Institute of Medicine’s guidelines for water intake for kids ages 1-13 years-old.

AGE GROUP (Y)	1-3	4-8	9-13
MEAN AGE (Y)	3	5	10
N RECRUITED	2	28	16
N COMPLETED	2	28	16
WEIGHT (KG)	16.1 $\pm$ 1.6	21.7 $\pm$ 3.4	40 $\pm$ 10.0
HEIGHT (CM)	98.5 $\pm$ 6.4	117.2 $\pm$ 10.2	144 $\pm$ 11.6
BMI (KG/M <sup>2</sup> )	16.6 $\pm$ 0.5	15.7 $\pm$ 1.2	19.1 $\pm$ 2.9

**Criteria for participation.** The inclusion criteria was as follows: 1. Boys aged 3-13 years-old. 2. Children willing to collect urine samples and store them in room temperature. 3.

Children willing to avoid strenuous exercise on day of collection. The exclusion criteria was as follows: 1. Evidence of clinically relevant metabolic, cardiovascular, hematologic, hepatic, gastrointestinal, renal, pulmonary, endocrine or psychiatric history of disease, based on medical questionnaire. 2. Use of medication that interferes with water metabolism. 3. Surgical operation on digestive tract. 4. Regular drug treatment within 15 days prior to start of study 5. Not willing to refrain from exercise on the day before and during urine collection. 6. Unwilling to collect urine. 7. Inability to participate in the entire study. 8. Use of Diapers. 9. Enuresis of nappies during the day or night.

**Screening:** The subject and their parent or guardian came to the lab to meet with a researcher prior to the start of the protocol. Consent was obtained from the parent or guardian and a medical history form was completed. We had them complete a socioeconomic questionnaire that included information on their ethnic background, socioeconomic status, income range, and parental education levels. The weekend protocol was explained and instructions were given on how to record nutrition data in dietary and fluid diaries that were child-specific. Guidelines were given on how to collect urine. Ten containers and one large container were provided for the subject to collect urine. The containers were clean and clearly labeled.

**Weekend protocol:** The weekend collection of urine began on Sunday morning after the first morning void. The subject collected each individual urine samples into numbered containers and recorded the time of void. The last void that was collected was the first void Monday morning. The subject recorded all fluid intake in a fluid diary starting Saturday morning when the subject first awakened. All food intake was recorded in a separate food diary. Parents assisted their children in recording their food and fluid intake. The time, type of food and amount of food



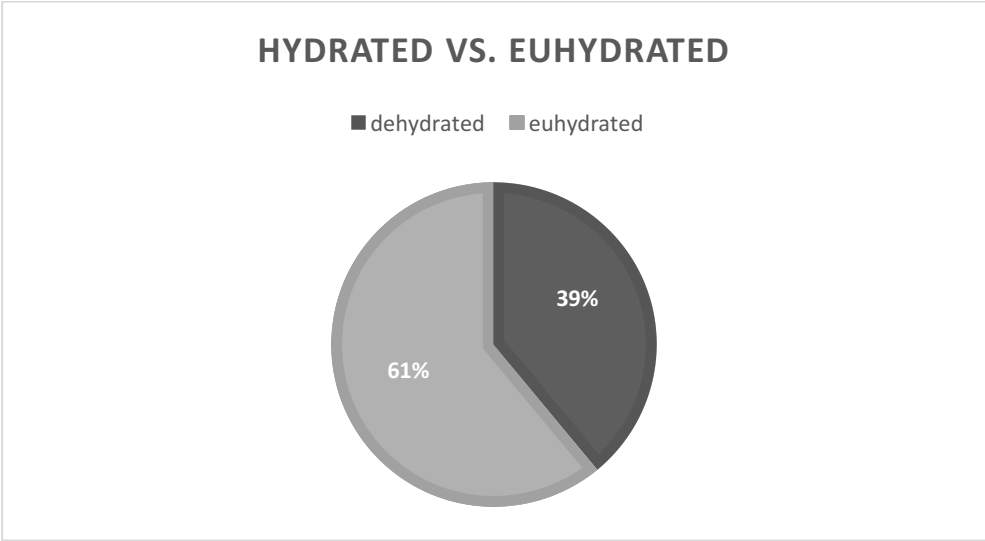
was all recorded in this diary. This record also began when the subject awakened Saturday morning. Fluid and food intake was recorded through Monday morning. Parents also helped children fill out an age appropriate IPAQ form. Children and parents kept track of physical activity on Saturday and Sunday.

**Urine Sample Analysis:** The 24-h sample of urine volume was weighed and recorded. After the volume was recorded, we analyzed the sample within 24-h for urine osmolality (uOsm), urine color (UC), and urine specific gravity (USG). Urine osmolality for each sample was measured in duplicates by freezing point depression (303 Advanced Osmometer, Advanced Instruments Inc, Norwood, MA, USA). The sample was measured a third time if the duplicates were different by  $\pm 5$  mOsm/kg. Inadequate hydration is defined by  $>800$  mOsm/kg. A hand-held refractometer (ATAGO SUR-NE, Tokyo, Japan) was used to measure the Urine Specific Gravity. Urine color was assessed while sample was in a glass tube and compared next to an 8-point urine color scale against a white background. Urine color was assessed by the same scientist, standing in the same room.

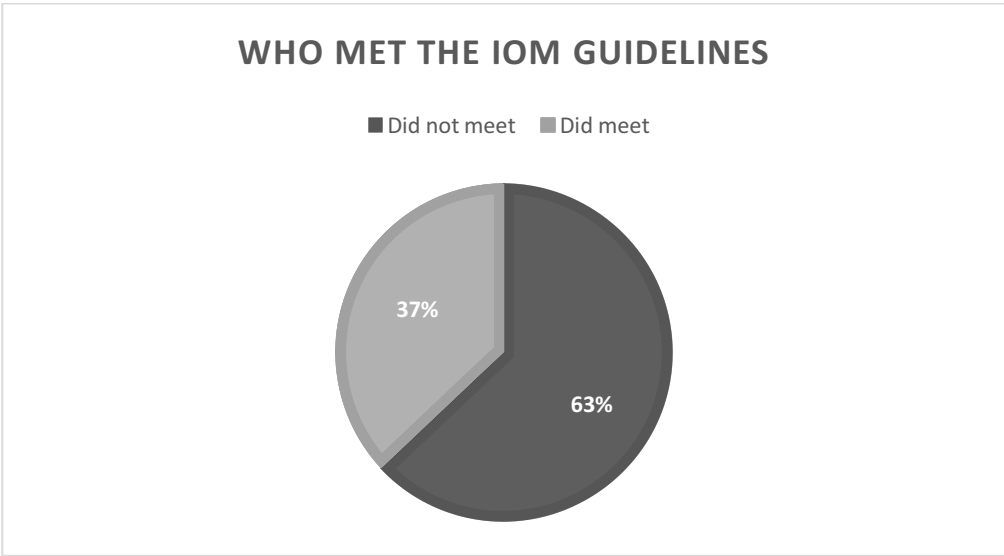
**Statistical Analysis:** All the raw data was compiled at the University of Arkansas. After all the data had been collected, two scientists entered the data into a personal computer that had data management software on it (JMP Pro 12). Outliers and extreme values were double checked with original data to ensure that there were not typing errors. Nutritional data was analyzed using Nutrition Data System for Research software (NDSR) by authorized scientists. Data analysis was checked by another scientist to check for errors. Fluid diaries were entered into a data sheet which calculated total water intake. This was also checked by a second scientists for errors. Data was analyzed using JMP statistical analysis software. Data is presented as mean  $\pm$  standard deviation.

## Results

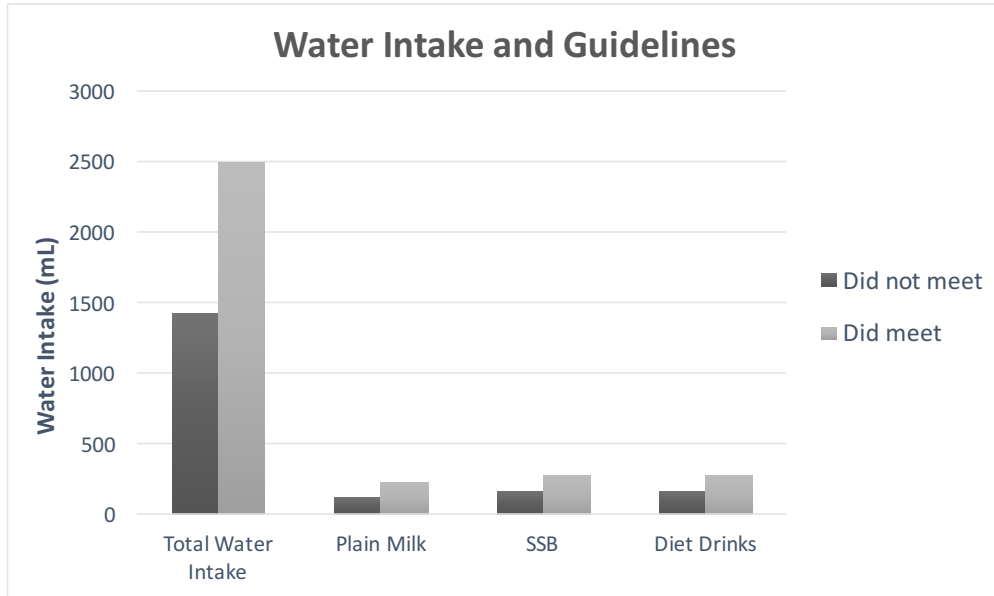
The analyzed sample was a group of 46 boys ages 3-13 years-old. There were eighteen boys out of the forty-six (39%) who were dehydrated (urine osmolality > 800 mOsm/kg) with an average urine osmolality of  $967 \pm 140$  mOsm/kg. Twenty-eight of the boys (61%) were euhydrated (urine osmolality < 800 mOsm/kg) with an average urine osmolality of  $530 \pm 150$  mOsm/kg. Twenty-nine of the boys (63%) did not meet the Institute of Medicine's (IOM) dietary guidelines for water intake for their age group. A t-test was run to determine if physical activity was different between dehydrated and euhydrated boys. Boys who met the dietary guidelines for water intake had much higher total water intake ( $2,501 \pm 706$  mL) compared to the boys who did not meet the guidelines ( $1435 \pm 408$  mL). Boys who met the guidelines had an average 24-h urine osmolality of 701 mOsm/kg. Boys who did not meet the guidelines had an average 24-h urine osmolality of 700 mOsm/kg. Physical activity was not different between dehydrated ( $5,506 \pm 4,941$  MET-min/wk) and euhydrated boys ( $4,575 \pm 4,338$  MET-min/week;  $P > 0.05$ ). Dehydrated boys had lower total water intake ( $1,661 \pm 759$  mL) than euhydrated ones ( $1,937 \pm 1,661$  mL). 24-h urine osmolality markers had a positive correlation with 24-h urine specific gravity and 24-h urine color.



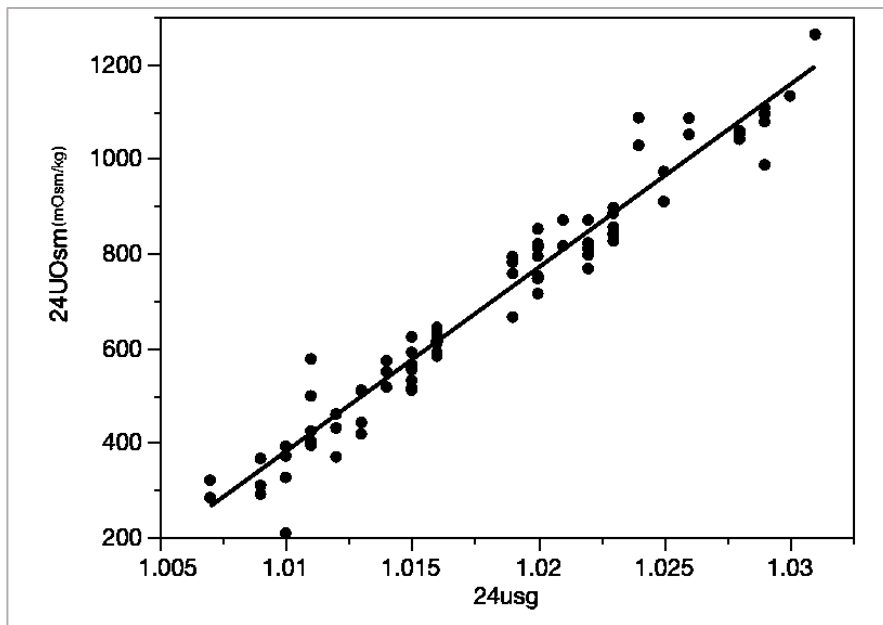
**Figure 1:** Percentage of forty-six boys who were hydrated and dehydrated.



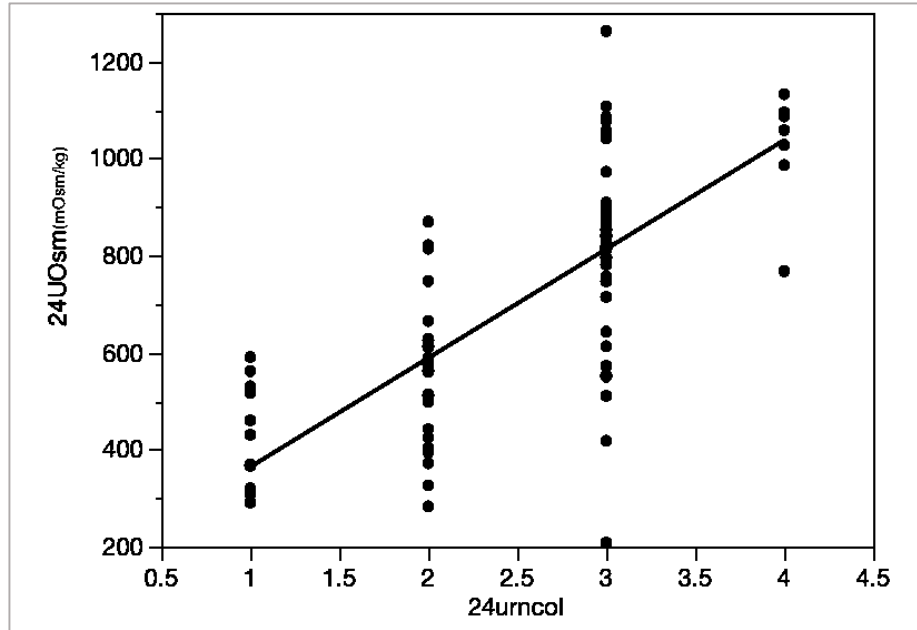
**Figure 2:** Percentage of forty-six boys who did meet or did not meet the Institute of Medicine's (IOM) guidelines for water intake.



**Figure 3:** Water intake compared between boys who did and did not meet the guidelines. SSB is short for sugar sweetened beverages. \*Values are presented as means.



**Figure 4:** Association between 24-h urine osmolality and 24-h urine specific gravity.  $R^2=0.98$  and  $P<0.001$ . \*Values for urine osmolality are expressed as mOsm/kg.



**Figure 5:** Association between 24-h urine osmolality and 24-h urine color.  $R^2=0.52$  and  $P<0.0001$ . \*Values for urine osmolality are expressed as mOsm/kg. Urine color is based on the original 8-color urine scale.

### Discussion

This is the first study in the United States to examine prevalence of dehydration in children by 24-h urine osmolality as the measure of hydration status. The purpose of this study was to examine hydration status in boys 3-13 years-old and their total water intake. The preliminary data collected thus far suggests that almost 65% of boys 3-13 years-old are not meeting the DRI's for water. In a previously published study, Drewnowski and his team examined fluid intake in 4,766 children 4-13 years-old. They did this by collecting 24-h recalls from NHANES in 3 separate surveys. What they found was that more than 75% of children, in all age groups, did not meet the DRI's for water (Drewnowski, 2013). A limitation of this study is that they relied on recalls, which is relying on subject's memory of what they had to eat and drink on the previous days. Our study relied on fluid and food intake diaries where subjects

recorded what they had to eat and drink through out there days, which doesn't rely on their memory.

Another study that was an NHANES survey was one that examined inadequate hydration among children in the US – specifically looking at differences in gender, race and ethnicity. This study had 4134 children participate between the ages of 6-19 years old. They also, like us, defined inadequate hydration as urine osmolality >800 mOsm/kg. They collected a spot urine sample for each participant and they also used a 24-hour recall survey by NHANES of all foods and beverages the participant had the day prior to urine collection. They found that nearly 55% of children were inadequately hydrated with higher urine osmolality observed in boys (Kenney, 2015). A couple major limitations of this study was that they used 24-h recalls, which are limiting for the same reasons stated above, but also that they relied on spot-urine samples. Spot-urine samples are not considered a reliable way to assess overall hydration status. A review conducted in 2015 by the U.S. Army Research Institute of Medicine concluded that spot-urine samples are often invalid and can give false positives and negatives when measuring hydration. Spot urine samples are the most convenient measurement, but often have higher urine osmolalities. The review also states that 24-hour urine sample is considered the best measurement for hydration assessment, acknowledging that this is not always convenient (Cheuvront, Kenefick, Zambraski, 2015). Other scientists such as Armstrong, Perrier and Kavouras have also conducted studies concluding that 24-hour samples are the most sensitive measurement of hydration status and it is considered the gold standard (Armstrong, 2013, Kavouras, 2002, Perrier, 2012). Knowing that this is considered the best way to assess hydration, our study is the first to use it in assessing dehydration prevalence in children in the US. However, 24-h urine samples were used in a study in 150 children in Greece ages 9-13 years-old.

This study also looked at water intake and hydration status. They found that 44% of boys and 23% of girls were hypohydrated (Kavouras, 2016). Our study has found that 40% of children are inadequately hydrated. Our prevalence is lower than the NHANES study, which we would expect due to having a more sensitive measurement of hydration.

We are finding that children are still not drinking enough fluid. Low water intake in school-aged children has been found to be associated with obesity, being overweight, and having a high BMI (Schwartz, 2016). Children who are obese have lower water intakes compared to normal weight children (Maffeis, 2015). Water intake is important for children's health, but they do not seem to be drinking enough water to be euhydrated. Most boys in our study did not meet the Institute of Medicine's dietary guidelines for water intake. Water intake data was collected by fluid and food diaries which averaged water intake over 48hrs. The Institute of Medicine breaks the 3-13 year-old age range into three categories, 1-3y, 4-8, 9-13. Interestingly, some boys that were hydrated also did not meet the guidelines. The boys who did meet the guidelines though, did have considerably higher total water intake. They drank more in plain water but also other types of beverages as well such as sodas, juices, and diet drinks. The average urine osmolality between those that did meet and those that did not meet the guidelines were not significantly different. This is not something that one would expect. Physical activity was not found to be significantly higher in those who did not meet the guidelines. It could also be due to having young mean age in each category. The children that participated in this study had low BMI's as well. The IOM guidelines for water intake is an average as well as a cut off. 20% of our boys who were considered hydrated (<800 mOsm/kg) did not meet the Institute of Medicine's guidelines. It's possible that for their size and age, they did not require as much water intake as the IOM guidelines suggest. As the study continues, and more data is collected, the

reason for this may become clear. We did not identify a reason for the similarity between urine osmolality's of these two groups.

The results of this study thus far are only preliminary results, with much more data collection in the future. We do acknowledge that there are still limitations to our study. The results so far are based on a small sample size. A small sample size is not representative of all children in the US. We also rely on participants to be honest when recording their nutrition data. We are aware that participants could choose or accidentally falsely record their nutrition intake. Also, those participating in this study know that they are participating in a nutrition study. Participants may change behavior due to this knowledge, drinking more than they typically would or making healthier choices. Participants are more aware of what they are eating and how much they are drinking due to having to record it throughout the two days. This could produce outliers in the data and cause us to not have accurate representation of their nutrition intake. That being said, because we are collecting their 24-hour urine samples – we can know that we are accurately assessing hydration status.

**In conclusion,** these preliminary data suggests that the majority of 3-13 year-old boys fail to meet the water intake recommendations and 4 out of 10 are hypohydrated. More data are needed to examine the factors that influence these observations.



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