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Determinants of Hydration in Children: The Role of Physical Activity and Parental Dietary Habits

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Determinants of Hydration in Children:
The Role of Physical Activity and Parental Dietary Habits

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by

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ABSTRACT

PURPOSE: The purpose of these studies was 1) to examine the factors that influence the water intake in children and 2) to identify the optimal time window to assess hydration status that would be equivalent to 24-hour urine sample in children. **METHODS:** Study 1: Data for 200 parents (age:3-13y, female:62%, BMI:28.4±7.0kg·m^{-2}) and 200 children (age:7.5±2.9y, female:44%, BMI:17.7±3.9kg·m^{-2}) were recruited. Subjects recorded their fluid and food consumption on the 2-day diary, and food data were analyzed by using the Nutrition Data System for Research (NDSR) program. Physical activity levels were assessed with the International Physical Activity Questionnaire (IPAQ). All urine samples were collected for 24-h. Study 2: Data for 541 children (age:3-13y, female:45%, BMI:17.7±4.0kg·m^{-2}) participated in the study. The equivalence of their mean of spot urine sample was tested compared to the 24-h urine sample to identify the optimal time window to measure the hydration status by using the mean of spot urine sample at specific time window [morning (0600-1159), early afternoon (1200-1559), late afternoon (1600-1959), evening (2000-2359), overnight (2400-0559), and first morning (0600-0959)]. **RESULTS:** In study 1, 59% of children did not meet the water intake guideline by Institute of Medicine (IOM) and 42% of them were underhydrated [24-h urine osmolality (UOsm):≥800mmol·kg^{-1}]. Children's age, BMI, total energy intake (TEI), total fat, total carbohydrate, total protein, and sodium intake were significantly associated with their water intake pattern, as well as parent's BMI, marital status and education (P<0.05). Fluid intake pattern was significantly correlated between child and parent (P<0.05). In study 2, mean of spot urine sample at late afternoon (1600-1959) showed an equivalent UOsm value compared to 24-h urine sample (P<0.05). The overall diagnostic ability of hydration based on the mean of spot urine sample at 1600-1959 was 91.3% AUC (area under the curve), and the threshold hold of 840 mmol·kg^{-1}. A moderate correlation was observed between the mean of spot UOsm at 1600-1959 and 24-h (R^2=0.657). **CONCLUSION:** These data suggest that various factors including parental practice influence children's water intake pattern. Moreover, late afternoon urine sample can be equivalent to assessing hydration status compared to the 24-h urine sample in children.

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I. INTRODUCTION

Water is a building material for cells, tissues, and organs (Lang, 2011) and it plays a vital role in our body. It helps the hydrolytic reactions for macronutrients (Jéquier & Constant, 2010) and it can be a solvent for protein synthesis (Haussinger, 1996). Total body water (TBW) can be obtained from fat-free mass (FFM), and it represents about 75 % of body weight in infants and decreases down to 55 % in older adults (Petraccia, Liberati, Giuseppe Masciullo, Grassi, & Fraioli, 2006; Wells, 2005). Although it is important to maintain fluid homeostasis by balancing inputs and outputs considering the amount of TBW, the importance of water often gets ignored due to its abundance in our daily life (Rush, 2013).

In the healthy human body, 5-10 % of TBW turns over every day to maintain fluid homeostasis (National Institutes of Health, 2005; Péronnet et al., 2012) but it may vary between children and adults when considered body surface area (Rush, Chhichhia, Kilding, & Plank, 2010). Human milk satisfies the daily water needs for infants according to the Institute of Medicine (IOM), however, daily water intake differ by age since TBW gradually decreases as we age (Raman et al., 2003).

The amount of water from food and endogenous metabolic production is not enough to meet daily water intake needs (Jéquier & Constant, 2010). Thus, IOM and European Food Safety Authority (EFSA) provides the guidelines for daily dietary water intake by gender and by different age group (EFSA, 2010; National Institutes of Health, 2005; Sebastian, Wilkinson Enns, Goldman, & Moshfegh, 2012). However, only 25% of children meet the water intake guideline in the United States (Drewnowski, Rehm, & Constant, 2013), and only 39% of children meet the water intake guideline all over the world (Ferreira-Pêgo et al., 2015). There can be various reasons affect these ubiquitous underdrinking phenomenon in children, but the associations have not been studied enough yet.

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II. STUDY 1

Determinants of Hydration in Children: The Role of Physical Activity and Dietary Habits

ABSTRACT

PURPOSE: The aim of the present study was to examine the factors that could influence the water intake behavior in children. **METHODS:** Data for 200 parents (age: 20-55 y, female: 62%, BMI: 28.4±7.0 $kg \cdot m^{-2}$) and 200 child (age: 3-13 y, female: 44%, BMI: 17.7±3.9 $kg \cdot m^{-2}$) were recruited from NorthWest Arkansas for the cross-sectional study. Subjects recorded their fluid [total water intake (TWI), plain water intake (PWI), total fluid intake (TFI), and sugar-sweetened beverage (SSB) intake] and food consumption on a 2-day diary from Saturday, and food data were analyzed by using Nutrition Data System for Research (NDSR) program. Physical activity was assessed with the International Physical Activity Questionnaire (IPAQ), and socioeconomic status (SES) was assessed during screening. All urine samples were collected for 24-h on a Sunday. **RESULTS:** Fifty-nine percent of children did not meet the water intake guideline by the Institute of Medicine (IOM) and 42% of them were underhydrated (24-h UOsm: ≥800 $mmol \cdot kg^{-1}$). Children's age, BMI, total energy intake (TEI), total fat, total carbohydrate, total protein, and sodium intake were significantly associated with their water intake pattern, as well as parent's BMI, marital status and education (P<0.05). Fluid intake pattern was significantly correlated between child and parent, and the significance got stronger after adjusted for body weight (P<0.05). **CONCLUSION:** These data suggest that various factors including parental practice influence children's water intake pattern.

Key Words: water intake, hydration status, children

INTRODUCTION

Underdrinking can lead to net fluid loss of body fluids and hypertonic hypovolemia, a condition known as dehydration (Cheuvront, Kenefick, Charkoudian, & Sawka, 2013; Costill, Coté, & Fink, 1976). Improper hydration state has been reported to be associated with chronic diseases and other health issues (Arnaoutis et al., 2017; Carroll, Davis, & Papadaki, 2015; Popkin & Rosenberg, 2010; Sontrop et al., 2013) such as obesity (Chang, Ravi, Plegue, Sonneville, & Davis, 2016) and impaired cognitive function (Ganio et al., 2011; Grandjean & Grandjean, 2007; Szinnai, Schachinger, Arnaud, Linder, & Keller, 2005). The adverse effect due to underdrinking may get more susceptible in children those who are more exposed to inadequate hydration (Masento, Golightly, Field, Butler, & Van Reekum, 2014; Maughan, 2012), and they can be improved simply by drinking more water (Turner & Hager, 2016). A cross-sectional study based on the national nutrition survey in children population (5-11 y; n=2,536) in Mexico reported that high consumption of plain water in combination with low consumption of sugar-sweetened beverage (SSB) was associated with less total energy intake (Shamah-Levy, García-Chávez, & Rodríguez-Ramírez, 2016). Another study reported that school performance in children was better after drinking 300 ml of water (Benton & Burgess, 2009). Although the beneficial effect of drinking water is evident, at least 75% of children do not meet the water intake recommendation by Institute of Medicine (IOM) (National Institutes of Health, 2005) and about 50% of children are underhydrated (urine osmolality: ≥ 800 mmol·kg^{-1}) in the US (Kenney, Long, Cradock, & Gortmaker, 2015). Children's diet and physical activity behavior are lifestyle factors that can be influenced by their parents (Jago et al., 2017; Lloyd, Lubans, Plotnikoff, & Morgan, 2015; Robinson, Rollo, Watson, Burrows, & Collins, 2015), however, the parental effect on their children's drinking water has not been explored yet.

Thus, the purpose of this study is to determine hydration in children by exploring various factors that could influence children's water intake and hydration status over the weekends.

METHODS

Participants

Two-hundred healthy children (3-13 y; boys and girls) and one of their parents (20-55 y; either mother or father) from the NorthWest Arkansas, who were willing to collect urine samples over a weekend, were participated in this cross-sectional study as a pair (Table 1). Both parents and children were excluded if they; 1) had evidence of clinically relevant metabolic, cardiovascular, hematologic, hepatic, gastrointestinal, renal, pulmonary, endocrine or psychiatric history of disease, based on the medical history questionnaire, 2) used medication that interferes with water metabolism, 3) had surgical operation on digestive tract, except possible appendectomy, 4) had regular drug treatment within 15 days prior to start of the study, 5) were unwilling to collect a urine sample, 6) had inability to participate in the entire study, or 7) were pregnant for females. Children were also excluded if they used a diaper or if they had enuresis or used nappies during the day or the night.

Screening for the Study

Qualified subjects, paired with parent and child, who passed the medical history check came into the Human Performance Laboratory at the University of Arkansas for the screening during the weekdays. Trained health professionals briefly went over the study protocol when they came in, and subjects were required to sign the consent form once they agree to participate in the study. After then, subjects filled out the questionnaire related to the ethnical background, socio-economic status (SES), and physical activity (international physical activity questionnaire; IPAQ) with the help of trained health professionals. Lastly, subject's height (including seating height) and weight were recorded on site after they had a brief toilet training to collect their urine samples in the urine containers. After the screening, subjects were sent home with a 2-day food and fluid diary and urine containers for urine collection. Urine hat was provided for girls to support their urine collection.

Study Protocol

Data collection for the study started on Saturday morning and subjects were asked to refrain from strenuous exercise during the experiment days. Pairs of child and parent were required to keep track of all their diets on both Saturday and Sunday and recorded them in the provided data sheets. Consumed beverages were recorded separately in the questionnaire specifically designed for fluid intake for children and adults, respectively (Johnson et al., 2017). Data collection for the urine sample started on Sunday. Subjects were asked to skip the first-morning urine void on Sunday and started to collect a sample from the second void in the provided numbered urine bottle until the first void of next morning as the first-morning sample on Monday would reflect the foods consumed on Sunday (Iglesia et al., 2016). Total of nine urine bottles was provided, and subjects collected urine samples in each bottle in order as they go. Void times were also recorded in the provided time sheet for all the voids over the weekend. Subjects used the provided additional orange urine container to collect the rest of urine voids if they exceed nine voids. Subjects were scheduled to drop off the sample on Monday, and each parent and child was rewarded with $50 gift card once they finish the study.

Laboratory Protocol

Trained health professionals retrieved the samples and the lab supplies at the Human Performance Laboratory on Mondays. After weighed individual urine voids in each urine bottle, 15 mL of samples were aliquoted in the glass tube for USG and urine color assessment as soon as they came in by using refractometer (Hand Refractometer, Atago, Tokyo, Japan) and urine color chart (Armstrong et al., 1994; Kavouras et al., 2016), respectively. After then, all the spot urine samples were mixed in the orange urine container for 24-h sample and also aliquoted for the analysis. All the spot urine sample and 24-h urine sample were transferred to the plastic tube and stored at 4℃ for the urine osmolality assessment by using osmometer (3250 Single-Sample Osmometer, Advanced Instrument, Norwood, MA, USA). Triplicates of 24-h samples were stored at -80℃ for any additional analysis. Fluid intake data including total water intake (TWI), total water intake from beverages (TWI-b), total fluid intake (TFI), plain water intake (PWI), and sugar-sweetened beverage (SSB) intake were obtained from the fluid intake questionnaire. Diet intake data were entered by using nutrition data system for research (NDSR) program (NDSR, version 2017, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA) and data for total water intake from foods (TWI-f), total energy intake (TEI), total carbohydrate (CHO), total fat, total protein, and sodium were obtained from NDSR. Recorded physical activity data were converted into METs per minute value (Ainsworth et al., 2011; IPAQ Research Committee, 2005).

Categorization of Variables

Total water intake in child and their parent were dichotomized into “meet” vs. “do not meet” based on the Adequate Intake (AI) level of water by IOM guideline (National Institutes of Health, 2005). AI by IOM guideline in children and adults are different by age and gender [3 y:

1,300 mL, 4-8 y: 1,700 mL, 9-13 y (girl): 2,100 mL, 9-13 y (boy): 2,400 mL, 20-55 y (female): 2,700 mL, 20-55 y (male): 3,700 mL]. Hydration status was also dichotomized into “adequately hydrated” vs. “underhydrated” by using urine osmolality (UOsm) cutoff of 800 $mmol \cdot kg^{-1}$ (Bar-David, Urkin, Landau, Bar-David, & Pilpel, 2009) and 500 $mmol \cdot kg^{-1}$ (Erica T. Perrier et al., 2015). SSB intake was dichotomized either consumed ≥ 1 cup (8 oz) vs. < 1 cup, or ≥ 2 cup (16 oz) vs. < 2 cup. Body mass index (BMI) for both children and parents was dichotomized into normal vs. overweight/obese. Children’s BMI was categorized as overweight at the 85^{th} to 94^{th} percentiles, and it was also categorized as obese above 95^{th} percentile based on the CDC growth charts (Centers for Disease Control and Prevention, 2000). Variables for SES were dichotomized as environmental factors not to sacrifice the degree of freedom; marital status [married/partnered vs. others (divorced/separated, single, widowed)], and education (above college graduate vs. less than college graduate).

Statistical Analysis

All variables are presented as a mean ± standard deviation and the average of Saturday and Sunday food and fluid intake value was used for the analyses. Association between water intake pattern in children and factors that could influence children’s water intake behavior were assessed using pairwise correlation and chi-square analysis. Variables that showed significant relationship with children’s fluid intake pattern were categorized if needed, and examined the association with each fluid intake components in children (TWI, TWI-b, TWI-f, TFI, PWI, and SSB intake) with both unadjusted and adjusted value [amount of fluid intake (mL) ÷ body weight (kg)]. Student’s t and ANOVA were used to examine the difference between the categorized groups. Generalized regression with log-normal distribution (because variables were positively skewed) was used to examine the contributing factors by adjusting for the covariates (age, gender, BMI, TEI, total CHO, total fat, total protein, sodium, physical activity) that have a linear relationship with children's water intake behavior. Lasso was used as an estimation method. The odds ratio of underhydrated children who do not meet the water intake guideline were obtained by using the chi-square test. The correlation coefficient for water intake between child and parent was examined with residual values after controlling the body weight. All statistical analyses were performed using JMP Pro (version 14.0, SAS Inc., Gary, NC, USA). A value of $P<0.05$ was regarded as statistically significant.

RESULTS

Total of 59% of children did not meet the water intake guideline based on TWI, while 48% of their parents were consuming water below the guideline by IOM (Figure 1). When dichotomized with 800 $mmol \cdot kg^{-1}$, 42% of children were underhydrated (≥ 800), while 79% of children were underhydrated with 500 $mmol \cdot kg^{-1}$ as the cutoff. Percentage of underhydrated population in parents were lower than children when used 800 $mmol \cdot kg^{-1}$ (13%), and it got more when used 500 $mmol \cdot kg^{-1}$ (46%). Fluid intake pattern in children were no different between underhydrated (≥ 800) and euhydrated (< 800) group with 800 $mmol \cdot kg^{-1}$ ($P>0.05$), however, euhydrated group in children had higher TWI, TWI-b, TFI, and PWI compared to underhydrated group with 500 $mmol \cdot kg^{-1}$ as cutoff ($P<0.05$). No variables for children's fluid intake were different between under- and euhydrated groups after adjusted for body weight ($P>0.05$). Urinary biomarkers in children were examined by TWI, and children who do not meet the IOM guideline had higher urine color compared to the children meet the guideline ($P<0.05$).

Factors Influence Children's Fluid Intake Behavior

There was no statistical difference by gender in children, while male had higher weight, BMI, TWI, TWI-b, TFI, 24-h UOsm, 24-h USG, TEI, total CHO, total fat, total protein, and sodium compared to females in an adult population ($P<0.05$). However, weight, height, BMI, TWI, TWI-b, TFI, PWI, SSB, TEI, total CHO, and sodium were statistically different in children when they divided into tertile by age (3 y, 4-8 y, 9-13 y; $P<0.05$; Table 1). TWI, TWI-b, TFI, and PWI in children increased as they age, however, the amount consumed rather decreased after adjusted for body weight ($P<0.05$). Only urine osmolality was lower in the age group 9-13 y compared to 4-8 y among urinary biomarkers ($P<0.05$). There was no statistical difference among race/ethnicity in children ($P<0.05$). Twenty-nine percent of children were overweight or obese, while 71% were normal when categorized by BMI (Table 2). Children who are overweight or obese had higher TWI, TWI-b, TFI, and PWI, however, the result got reversed when examined with adjusted value by body weight ($P<0.05$). Only urine osmolality was showed statistical significance among urinary biomarkers, higher in the normal group ($P<0.05$).

In the current study, children are drinking an average of one serving of SSB (8 oz = 237 mL) per day (18% of TFI). Moreover, 36% of children consumed more than one cup of SSB, and 21% of children consumed more than two cups of SSB. Percentage of parents who consumed SSB were higher (≥1 cup: 47%, ≥2 cup: 32%) compared to children (Table 2). Children in the group that consumed more SSB (either ≥1 cup or ≥2 cup) also had higher TWI, TWI-b, and TFI ($P<0.05$), however, there was no difference in urinary biomarkers in both groups that have a different level of SSB consumption ($P>0.05$). Physical activity in children was normally distributed and SSB intake was also different by physical activity level. Children with a low level of physical activity consumed more SSB (adjusted with kg of body weight) only compared to the group with moderate physical activity level ($P<0.05$).

Parental Influence on Fluid Intake Behavior in Children

Children's fluid intake behavior (TWI, TWI-f, TWI-b, TFI, PWI, and SSB) intake were significantly correlated with parent's fluid intake behavior either unadjusted or adjusted for body weight ($P<0.05$). As a physiological factor, parent's BMI also had affect on their children's fluid intake pattern. Children had higher TWI-b, TFI, and SSB intake if they have parents with overweight/obese ($P<0.05$), however, the difference diminished when examined with adjusted values (mL/kg) ($P>0.05$). Children's physical activity level was correlated with their parent's physical activity level ($P<0.05$). Children who have the parents with low or high physical activity level had higher TWI, TWI-b, TFI, and PWI compared to parents with moderate physical activity level ($P<0.05$). Parent's marital status and education also influenced their children's fluid intake pattern. Children with married/partnered parent had lower TWI-b and SSB intake compared to another marital status, and the urine color in this the same group was lower ($P<0.05$). Children who have parents with an education above college graduate also had lower TFI and SSB intake compared to parents with education less than college graduate ($P<0.05$).

Lastly, generalized regression showed that all the children's fluid intake pattern differed by age except for TWI-f and PWI after adjusted for gender, BMI, TWI, sodium, total fat, total CHO, total protein, physical activity, parent's marital status, and parent's education ($P<0.05$; Table 3). BMI contributed in explaining most of the water intake behavior (TWI, TWI-b, TFI, PWI) after controlling for other covariates ($P<0.05$). Urine osmolality and USG also showed a negative association with BMI after adjusting other covariates, but they were not statistically significant ($P>0.05$). Total protein and sodium contributed in explaining TWI, TWI-f, and PWI, while parent's marital status and education contributed in explaining TFI and SSB consumption (*P*<0.05). Parent's marital status was also associated with their children's urine color after adjusting other covariates (*P*<0.05).

DISCUSSION

In the current study, 200 children from NorthWest Arkansas participated and found that 59% of them did not meet the AI of water by IOM recommendation and 42% of them were underhydrated (≥ 800).

Determinants of Water Intake Behavior in Children

Although water often neglected as a nutrient (Rush, 2013), it is essential in our body and the daily water needs are different as we age (Rush et al., 2010; Sawka, Cheuvront, & Carter, 2005) and it was consistent in the current study. The total amount of fluid intake in children was higher in the older children compared to the younger children. This difference between different age group got reversed when adjusted by body weight. It can imply that children are not drinking enough as they age, even though, the total amount of fluid intake increases as they age. In addition to aging, there are various factors that could influence drinking water behavior.

Inadequate hydration is reported to be associated with increased TEI and obesity (Chang, Ravi, Plegue, Sonneville, & Davis, 2016; Daniels & Popkin, 2010; Tate et al., 2011; Thornton, 2016). The current study showed that children in the overweight or obese group consumed more beverages including more plain water with lower urine osmolality compared to the normal weight group. However, the result got reversed when examined with adjusted values by body weight, while PWI stayed the same between groups. This can be explained that children in the overweight or obese group seem to consume more fluid which allowed to have a lower urinary marker. However, this may be driven by relative higher beverage intake other than plain water in the normal weight group when considering their body weight as PWI stayed the same between the two groups. There are some previous studies also showed the association between children hydration and obesity status. One study examined the association between obesity and hydration biomarkers in children (7-11 y) in Italy, and they found that 24-h urine osmolality was higher in the obese children compared to normal weight children ($P<0.05$) (Maffeis et al., 2016). A Recent study in *JAMA Pediatrics* also showed the positive influence of increasing water access on child obesity (Schwartz, Leardo, Aneja, & Elbel, 2016). They examined 1,065,562 public elementary school students in New York and found a significant reduction in body mass index (BMI) by improving access to water via the installation of water jets (electrically cooled, large clear jugs with a push lever for fast dispensing). Placing water dispensers at lunchtimes in school cafeterias can be a cost-effective intervention that could prevent 0.5 million cases of childhood overweight and the lifetime cost saving total can be up to $13.1 billion (net benefit of $174 per student) nationwide (An, Xue, Wang, & Wang, 2017).

Numerous evidence supports that consumption of SSB is positively associated with the prevalence of obesity (Hu, 2017). It is also reported that replacing SSB consumption with water would be beneficial on TEI, obesity, and healthy fluid intake (Duffey & Poti, 2016), and it is consistent with children population (Shamah-Levy et al., 2016). The current study showed that children who consumed more SSB also showed the trend to consume more beverages in general. However, the result showed that the amount of SSB consumption did not influence children's hydration status based on the urinary biomarkers.

Amount of sweat produced increases with the intensity of the exercise as well as with the ambient temperature and humidity (Sawka et al., 2007). It is important to replenish body fluid according to the amount of sweat loss to prevent dehydration during exercise since it could lead to detrimental effect due to increased physiological strain (McDermott et al., 2017). Thus, the level of physical activity maybe plays a role in explaining water intake in children. As we expected, children's physical activity showed some relationship with their fluid intake behavior. Physical activity level increased in children as they age in the current study, however, only SSB (adjusted by body weight) had difference according to the physical activity level. Children in the low level of physical activity group consumed more SSB compared to the group that has moderate physical activity level.

Parental Influence on Children's Fluid Intake Behavior

Drinking water in children is a lifestyle behavior that can be influenced by their parents. For instance, parental lifestyle has been reported to be associated with their children's behavior, diet (Robinson et al., 2015), and physical activity (Jago et al., 2017; Lloyd et al., 2015). A meta-analysis showed that parental guidance affected both healthy and unhealthy food consumption (Yee, Lwin, & Ho, 2017), while a longitudinal study showed that parental behavior, but not the verbal pressure, influenced the diet and physical activity of their children (Zarychta, Mullan, & Luszczynska, 2016). This also applies to create a drinking water habit. Mantziki and her colleagues examined 1,187 kids from seven European communities and showed that strict parenting practice towards soft drinks had a favorable influence on water intake in children (Mantziki, Renders, & Seidell, 2017). Water intake was also increased in both children and parents in a family-setting intervention focused on water intake (Franks, Lahlou, Bottin, Guelinckx, & Boesen-Mariani, 2017; Lahlou, Boesen-Mariani, Franks, & Guelinckx, 2015).

The current study also had the similar results showing that children's fluid intake behavior was correlated with parent's fluid intake behavior either unadjusted or adjusted for body weight. Especially, children who have parents with overweight or obese consumed more

TWI-b and TFI including SSB intake compared to the children who had parents with normal weight based on their BMI. As shown earlier, parent's marital status and education also affected children's fluid intake pattern. When children have a married /partnered parents or parent's with a higher education above college graduate, they tend to consume less SSB and TWI-b, and they also tend to have lower urine color after adjusted for body weight.

Stimulation of thirst that represents the elevated arginine vasopressin (AVP) (Hughes, Mythen, & Montgomery, 2018) has been considered less important due to the abundance of water access in our daily life. Moreover, people who are physically active consume more water these days for their well-being according to the change in lifestyle (Rosinger & Herrick, 2016). A recent study that examined the association between physical activity and beverage consumption in adolescents (n=1,988; 12-17 y) reported that girls who are physically active had a higher mean of daily water (del Mar Bibiloni, Özen, Pons, González-Gross, & Tur, 2016). Another study examined the food and beverage intakes in 7,229 children (2-10 y) from eight European countries according to different physical activity levels and found that, boys who spent less time in moderate-to-vigorous physical activity (MVPA) were more likely to consume water compared to those in the highest MVPA tertile ($P<0.05$) (Santaliestra-Pasías et al., 2018). The current study showed that children's physical activity level was influenced by their parent's physical activity level as they were correlated with each other. Similarly, parents with a high level of physical activity had a trend to consume more beverages compared to the group with the moderate level of physical activity. However, the interpretation can be controversial because the group with the low level of physical activity also had higher fluid consumption compared to the group with the moderate level of physical activity. This inconsistency may be due to the discrepancy of days (weekend vs. 7-days) for data collection as IPAQ is designed to collect the data of past 7-days (Ainsworth et al., 2011).

There were various factors that could influence the hydration in children in the present study. Generalized regression analysis showed that part of water intake behavior in children was explained by age for the most of fluid intake pattern. BMI explained the part of the water intake component only and the hydration status in children, showing that hydration status in children is associated with the obesity status. Protein and sodium are the components that could influence the osmotic load in our body (National Institutes of Health, 2005) and they also contributed to explaining water intake component in the current study as well. Lastly, the consumption of beverages including SSB was influenced by parent's marital status and parent's education. This may imply that children in the stable family environment would have better hydration status.

This cross-sectional study has enough sample size to draw out a proper conclusion in explaining the factors that could contribute hydration in children, yet, this study still has some limitations. Various components need to be considered to assess water influx such as metabolic, inspiratory, transcutaneous, and water consumption (Rush et al., 2010). Estimating the intake of water by using self-reported dietary data in this study can be controversial (Bardosono et al., 2015). Because age, gender, climate, and physical activity still needs to be considered when measuring water influx (Jéquier & Constant, 2010) and accessibility of fluid intake questionnaire for children is still limited (Rush et al., 2010). However, this method still can provide information on food intake, behavior, and eating patterns that other biomarkers cannot provide (Subar et al., 2015). Measuring the physical activity behavior via IPAQ also can be a limitation. Thus, measuring energy expenditure and total body water by using doubly labeled water on the day of the experiment can be an alternative solution for future studies (Bila et al., 2017; Nagy, 1983).

In conclusion, hydration in children is a complex phenomenon that can be determined by the combination of various factors. Children’s fluid intake increases as they age, however, they may be rather drinking less considering their body weight. Overweight or obese children may drink more fluids, however, this difference can be driven by SSB consumption and their hydration status was not different compared to normal children. Children’s water intake pattern is correlated with their parent’s even after adjusted for body weight, and they showed a similar trend when divided by physical activity level. Parent’s marital status and education also affected children’s water intake behavior, implying that children would be more adequately hydrated if they have the stable family environment.

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APPENDIX

Table 1. Basic characteristics

	Child												Parent		
	3 y			4-8 y			9-13 y			Total			Total		
	Female (n=4)	**Male (n=6)**	**All (n=10)**	**Female (n=47)**	**Male (n=67)**	**All (n=114)**	**Female (n=36)**	**Male (n=40)**	**All (n=76)**	**Female (n=87)**	**Male (n=113)**	**All (n=200)**	**Female (n=124)**	**Male (n=76)**	**All (n=200)**
Age (y)	3.0±0.0	3.0±0.0	3.0±0.0	5.8±1.3	5.9±1.4	5.9±1.4	10.5±1.4	10.7±1.5	10.6±1.4	7.6±2.8	7.5±2.9	7.5±2.9	36.0±6.2	37.0±5.9	36.4±6.1
Weight (kg)	15.8±2.0	15.7±2.6	15.7±2.3	22.2±5.1	23.6±5.9	23.1±5.6	44.7±12.3	46.2±16.0	45.5±14.4*†	30.9±14.2	31.1±15.4	31.0±14.9	76.7±21.4	90.3±21.1‡	81.9±22.2
Height (cm)	95±3	100±6	98±6	118±11	120±13	119±12*	148±9	150±13	149±11*†	129±19	129±20	129±19	165±7	177±7‡	169±9
BMI (kg·m^2)	17.5±1.3	15.7±1.2	16.3±1.5	15.9±1.8	16.3±1.9	16.1±1.8	20.1±3.8	20.0±4.0	20.0±3.9*†	17.6±3.4	17.7±4.2	17.7±3.9	28.3±7.5	28.7±6.2	28.4±7.0
TWI (mL)	1,372±356	1,573±860	1,493±681	1,524±645	1,686±684	1,619±670	2,045±688	2,182±846	2,117±773*†	1,732±701	1,855±786	1,802±751	3,071±1,077	3,590±1,198‡	3,268±1,149
TWI-f (mL)	394±234	634±353	538±320	447±198	457±259	453±235	521±216	509±240	515±227	476±208	485±259	481±238	622±259	714±301‡	657±279
TWI-b (mL)	977±287	939±925	955±709	1,076±550	1,229±617	1,166±592	1,524±690	1,673±744	1,602±718*†	1,257±640	1,371±714	1,321±683	2,449±1,038	2,875±1,135‡	2,611±1,093
TFI (mL)	1,042±336	967±936	997±725	1,153±588	1,297±638	1,238±619	1,608±707	1,774±778	1,695±745*†	1,336±667	1,448±744	1,400±712	2,520±1,048	2,976±1,158‡	2,693±1,111
PWI (mL)	439±293	687±812	588±641	555±417	713±475‡	648±457	928±709	972±598	951±649†	704±581	804±550	760±564	1,461±1,000	1,642±1,083	1,530±1,033
SSB (mL)	133±170	15±36	62±119	191±280	187±266	189±270	349±412	383±390	367±398*†	254±344	247±326	250±333	305±394	515±660‡	385±520
Alcohol (mL)	.	.	.	.	.	.	.	.	.	.	.	.	141±252	215±379	169±308
24-h UOsm (mmol·kg^{-1})	637±175	746±138	702±155	772±212	727±244	745±232	650±256	696±234	674±244	715±236	717±235	716±235	488±211	545±219‡	510±216
24-h USG	1.018±0.005	1.020±0.004	1.019±0.004	1.020±0.005	1.019±0.006	1.020±0.006	1.018±0.006	1.019±0.006	1.018±0.006	1.019±0.006	1.019±0.006	1.019±0.006	1.013±0.006	1.015±0.006‡	1.014±0.006
24-h UCol	3.0±1.6	3.0±0.6	3.0±1.1	2.9±0.8	2.6±0.9‡	2.7±0.8	2.6±1.0	2.8±0.9	2.7±1.0	2.8±0.9	2.7±0.9	2.7±0.9	2.4±0.9	2.5±0.9	2.4±0.9
TEI (kcal)	1,486±396	1,380±458	1,422±415	1,564±557	1,540±457	1,550±498	1,780±481	1,773±591	1,776±538†	1,650±527	1,614±519	1,630±521	1,77±489	2,148±593‡	1,917±558
Total CHO (g)	186.8±73.4	186.3±52.8	186.5±57.8	194.5±58.1	193.5±62.2	193.9±60.3	225.0±75.7	217.6±76.4	221.1±75.7†	206.8±67.6	201.7±67.7	203.9±67.5	196.5±67.5	232.2±80.9‡	210.1±74.7
Total Fat (g)	63.1±9.7	52.6±23.2	56.8±18.9	66.6±43.8	63.4±25.8	64.8±34.3	73.4±19.8	75.7±29.2	74.6±25.1	69.3±34.7	67.2±27.6	67.1±30.8	81.2±27.5	96.4±32.3‡	87.0±30.2
Total Protein (g)	48.1±13.1	48.3±16.3	48.2±14.3	52.0±17.1	54.8±22.3	53.7±20.3	59.8±18.4	61.2±25.4	60.5±22.2	55.0±17.8	56.8±23.3	56.0±21.1	71.3±22.0	91.0±28.5‡	78.8±26.4
Sodium (g)	2.4±0.7	2.0±0.7	2.1±0.7	2.6±1.0	2.5±0.8	2.5±0.9	3.0±0.8	3.2±1.2	3.1±1.1*†	2.74±0.9	2.73±1.0	2.74±1.0	3.26±1.05	3.96±1.18‡	3.53±1.15
PA (MET-min·wk^{-1})	777±0	1,559±1,081	1,428±1,018	2,298±1,768	2,845±2,128	2,585±1,973	2,478±1,859	2,801±1,989	2,650±1,920	2,348±1,791	2,750±2,031	2,565±1,929	2,650±1,455	2,833±1,378	2,716±1,426

BMI, body mass index; TWI, total water intake; TWI-f, total water intake from foods; TWI-b, total water intake from beverages; TFI, total fluid intake; PWI, plain water intake; SSB, sugar-sweetened beverages; UOsm, urine osmolality; USG, urine specific gravity; UCol, urine color; TEI, total energy intake; CHO, carbohydrate; PA, physical activity
*Significant difference between gender (P<0.05)

Table 2. Sugar-sweetened beverages intake, BMI, and physical activity category by age group and by gender in child and parent

		Child										Parent		
		3 y			4-8 y			9-13 y			All	20-55 y		
		Girl	Boy	All	Girl	Boy	All	Girl	Boy	All		Female	Male	All
SSB 1 cup														
< 1 SSB	%	75	100	90	72	70	71	58	45	51	65	72	64	174
≥ 1 SSB	%	25	0	10	28	30	29	42	55	49	36	52	12	25
SSB 2 cup														
< 2 SSB	%	100	100	100	83	87	85	69	65	67	79	90	34	107
≥ 2 SSB	%	0	0	0	17	13	15	31	35	33	21	34	42	92
BMI														
Normal	%	33	100	78	71	79	76	66	59	62	71	41	25	35
Overweight/Obese	%	67	0	22	29	21	24	34	41	38	29	59	75	65
Physical Activity														
Low	%	0	20	17	15	11	13	10	6	8	11	16	15	16
Moderate	%	100	60	66	51	47	49	61	53	57	53	42	40	41
High	%	0	20	17	34	42	38	29	41	35	36	42	45	43

SSB, sugar-sweetened beverage; BMI, body mass index

Table 3. Generalized regression with log-normal distribution to examine the contributing factors on children's fluid intake behavior

Hydration in Children	**Contributing Factors**	R^2	***P*-value**
Fluid Intake			
TWI	**Age** (+), **BMI** (+), **total protein** (+)	0.39	<0.05
TWI-f	TEI (-), total fat (+), total CHO (+), **total protein** (+), sodium (+)	0.56	<0.05
TWI-b	**Age** (+), **BMI** (+)	0.32	<0.05
TFI	**Age** (+), **BMI** (+), education (+)	0.39	<0.05
PWI	**BMI** (+), **total protein** (+)	0.25	<0.05
SSB	**Age** (+), marital status (+), education (+)	0.35	<0.05
Hydration Status			
UCol	Marital status (+)	0.13	0.012

Adjusted for age, gender, BMI, TEI, total CHO, total fat, total protein, sodium, physical activity, marital status, education

Note: R^2 in this table is for the global effect, and all the listed contributing factors were statistically significant

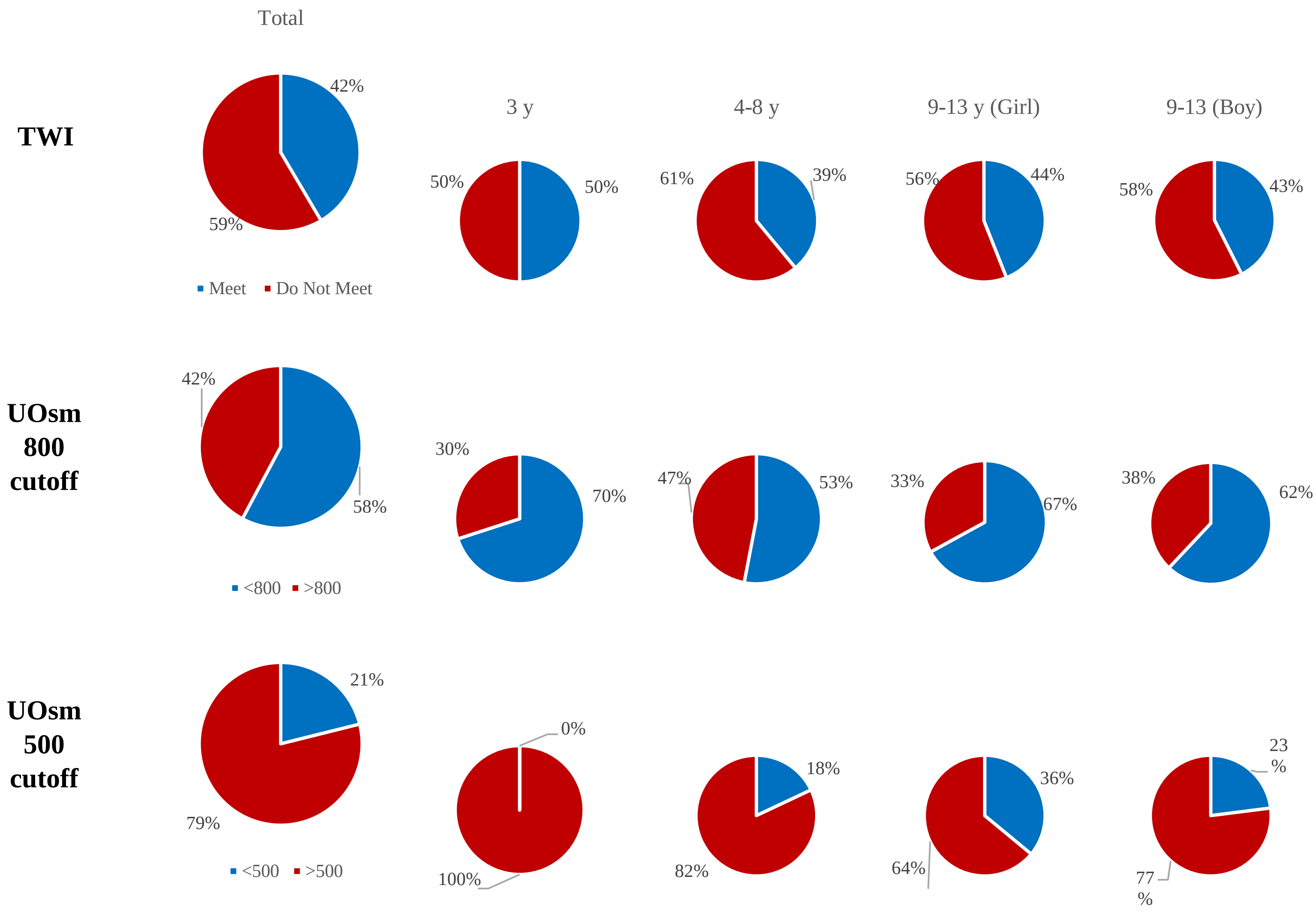
Bold: variables that appeared more than three times across the water/fluid intake data

+: positive association, -: negative association

FIGURE LEGENDS

Figure 1. Total water intake by IOM guideline and hydration status by UOsm cutoffs in children

Figure 1.



Total
TWI
42%
59%
Meet
Do Not Meet
3 y
50%
50%
4-8 y
61%
39%
9-13 y (Girl)
56%
44%
9-13 (Boy)
58%
43%
UOsm 800 cutoff
42%
58%
<800
>800
30%
70%
47%
53%
33%
67%
38%
62%
UOsm 500 cutoff
21%
79%
<500
>500
0%
100%
18%
82%
36%
64%
23 %
77 %

III. STUDY 2

Equivalence Test of Spot Urine Sample for Hydration Status Assessment Compared to 24-h Urinary Biomarker in Healthy Children

ABSTRACT

PURPOSE: The purpose of these studies was to identify the optimal time point to measure the hydration status compare to 24-hour urine sample in children. **METHODS:** Data for 541 children (age: 3-13 y, female: 45%, BMI: 17.7±4.0 kg·m^{-2}) participated in the study. The equivalence of their spot urine sample was tested compared to the 24-h urine sample to identify the optimal time point to measure the hydration status by using spot urine sample. Subjects recorded their fluid and food consumption in the 2-day diary. Spot urine sample was collected in a separate urine collection bottle throughout a day, and they were combined all together into the 24-h urine collection container to obtain the 24-h urine sample. Mean value of spot urine sample within specific time window [morning (0600-1159), early afternoon (1200-1559), late afternoon (1600-1959), evening (2000-2359), overnight (2400-0559), and first morning (0600-0959)] were compared with 24-h urine sample for the different analyses. **RESULTS:** Late afternoon spot urine sample (1600-1959) showed an equivalent UOsm value compared to 24-h urine sample ($P<0.05$). The overall diagnostic ability of hydration based on the late afternoon mean of spot urine sample value was 91.3% AUC (area under the curve) with sensitivity: 82.5% and specificity: 88.1%, and the threshold hold of 840 mmol·kg^{-1}. A moderate correlation was observed between the mean of spot UOsm at late afternoon (1600-1959) and 24-h UOsm (R^2=0.657). **CONCLUSION:** These data suggest that late afternoon urine sample around 1600-1959 can be equivalent on assessing hydration status compared to 24-h urine sample when 24-h urine collection is not available in large-sample size studies.

Keywords: spot, 24-h urine sample, hydration assessment, children

INTRODUCTION

About 55-75% of the human body consists of water, and it plays a vital role as a building material for cells, tissues, and organs (Lang, 2011). Water also can be a solvent for protein synthesis (Haussinger, 1996) and it helps the hydrolytic reactions for macronutrients (Jéquier & Constant, 2010) and thermoregulation (McKinley, Martelli, Pennington, Trevaks, & McAllen, 2018; Sawka, Cheuvront, & Kenefick, 2015). Precise hydration biomarkers assessment is important to maintain fluid homeostasis by balancing inputs and outputs considering the amount of total body water (Perrier, 2017). Although spot urine sample is not recommended for hydration assessment (Cheuvront, Kenefick, & Zambraski, 2015) due to its fluctuation throughout the day (Perrier, Demazières, et al., 2013), it has been used to assess the hydration status in children (Bar-David, Urkin, Landau, Bar-David, & Pilpel, 2009; Bonnet et al., 2012; Gouda et al., 2015; Kenney et al., 2015; Michels, Van den Bussche, Vande Walle, & De Henauw, 2017; Stookey, Brass, Holliday, & Arieff, 2012). There have been attempts to validate spot urine in predicting 24-h sodium excretion (Ji et al., 2012; Zhou et al., 2017), however, it is still controversial. Recent study examined the association between spot and 24-h urinary hydration biomarkers in free-living healthy adults, and they found that urine osmolality and USG values obtained from the void between 1400 and 2000 hours (mid- to late-afternoon) were equivalent to the 24-h urine sample (Bottin, Lemetais, Poupin, Jimenez, & Perrier, 2016). However, the validation study of spot urine sample comparing with 24-h sample has not been done in children population yet. Thus, the purpose of this study is to identify the optimal time window in children that is equivalent to 24-h urine sample for practical hydration assessment in our daily life.

METHODS

Participants

Five-hundred and forty-one healthy children (3-13 y; 45% girls) from NorthWest Arkansas participated in this cross-sectional study (Table 1). Children were excluded if they used a diaper or if they had enuresis or used nappies during the day or the night. Subjects were also excluded if they; 1) had evidence of clinically relevant metabolic, cardiovascular, hematologic, hepatic, gastrointestinal, renal, pulmonary, endocrine or psychiatric history of disease, based on the medical history questionnaire, 2) used medication that interferes with water metabolism, 3) had surgical operation on digestive tract, except possible appendectomy, 4) had regular drug treatment within 15 days prior to start of the study, 5) had an inability to participate in the entire study, or if the family were unwilling to collect urine sample.

Screening for the Study

Qualified subjects who passed the medical history check came into the Human Performance Laboratory at the University of Arkansas with their parent for the screening during the weekdays. Trained health professionals briefly went over the study protocol when they came in, and subjects were required to sign the consent form once they agree to participate in the study. After then, subjects had a brief toilet training to collect their urine samples in the urine containers. After the screening, subjects were sent home with a 2-day fluid diary and urine containers for urine collection. Urine hat was provided for girls to support their urine collection.

Study Protocol

Data collection for the study started on Saturday morning and subjects were asked to refrain from strenuous exercise. Subject's parents were required to help their child to keep track on all the fluid intake of their child for two days and recorded them in the questionnaire specifically designed for fluid intake for children (Johnson et al., 2017). Data collection for the urine sample throughout a day. Subjects were asked to skip the first-morning urine void on the first day and started to collect a sample from the second void in the provided numbered urine bottle until the first void of next morning, because the first-morning sample on the next would reflect the foods consumed on the day before (Iglesia et al., 2016). Total of nine urine bottles was provided, and subjects collected urine samples from each bottle in order as they go with the help of their parents. Void times were also recorded in the provided time sheet for all the voids over the weekend. Subjects used the provided additional orange urine container to collect the rest of urine voids if they exceed nine voids. Subjects were scheduled to drop off the sample on Monday, and the family was rewarded with $50 gift card once they finish the study.

Laboratory Protocol

Trained health professionals retrieved the samples and the lab supplies at the Human Performance Laboratory on Mondays. After weighed individual urine voids in each urine bottle, 15 mL of samples were aliquoted in the glass tube for USG and urine color assessment as soon as they came in by using refractometer (Hand Refractometer, Atago, Tokyo, Japan) and urine color chart (Armstrong et al., 1994; Kavouras et al., 2016), respectively. After then, all the spot urine samples were mixed in the orange urine container for 24-h sample and also aliquoted for the analysis. All the spot urine sample and 24-h urine sample were transferred to the plastic tube and stored at 4℃ for the urine osmolality assessment by using osmometer (3250 Single-Sample Osmometer, Advanced Instrument, Norwood, MA, USA). Triplicates of 24-h samples were stored at -80℃ for any additional analysis. Fluid intake data including total fluid intake (TFI), plain water intake (PWI), and sugar-sweetened beverage (SSB) intake in children were obtained from the fluid intake questionnaire.

Statistical Analysis

Distribution analysis was presented in means and standard deviations in Table 1. The mean of urine osmolality at each time window [morning (0600-1159), early afternoon (1200-1559), late afternoon (1600-1959), evening (2000-2359), overnight (2400-0559), and first morning (0600-0959)] was compared with 24-h urine osmolality for the different analyses. Mixed model multiple comparisons for a mean of spot urine sample was used to identify the specific time window that is not significantly different from a 24-h urine sample. Dunnett's was used to perform the comparisons by setting mean of 24-h urine sample as control level. Equivalence between the mean of spot urine sample at each time window and a 24-h urine sample was also tested by using urine osmolality. Mean absolute difference of urine osmolality between the mean of spot- and a 24-h urine sample was calculated with the lower- and upper-limit of their confidence intervals (CI). In this equivalence test, mean of spot urine osmolality at each time window were tested to see if both the urine osmolality of mean absolute difference and its CI fell below the bounds for equivalence set *a priori* (± 100 $mmol \cdot kg^{-1}$) obtained from a previous study (Bottin et al., 2016). The bound of ± 100 $mmol \cdot kg^{-1}$ not only represents 10% of the normal range of urine concentration but also considered precise enough to discriminate habitual low vs. high drinkers (Armstrong et al., 2010; Perrier, Vergne, et al., 2013). The pairwise correlation was performed between the mean of spot urine sample on the identified time window and 24-h urine sample. Receiver operating characteristic (ROC) curve was used to identify the threshold of the mean of spot urine osmolality with dichotomized 24-h urine osmolality by using cutoffs (800 $mmol \cdot kg^{-1}$) (Bar-David et al., 2009). Sample size at each time window was different as the children did not void at every time window. TFI was dichotomized into meet vs. do not meet the 80% of total water intake (TWI) guideline by Institute of Medicine (IOM) (National Institutes of Health, 2005) as TFI is considered as 80% of TWI (Kavouras et al., 2017). All statistical analyses were performed using JMP Pro (version 14.0, SAS Inc., Gary, NC, USA). A value of $P<0.05$ was regarded as statistically significant.

RESULTS

Total of 541 children (age: 3-13 y, girls: 45%, BMI: 17.7±4.0) were participated in this cross-sectional study (Table 1). Fifty-two percent of children did not meet the water intake guideline by IOM for Adequate Intake (AI). Thirty-seven percent of children were underhydrated with urine osmolality of 800 $mmol \cdot kg^{-1}$ as a cutoff. There were significant differences in most of the variables by age except for the urine color (P<0.05).

The result of mixed model multiple comparisons showed that urine osmolality of all the mean of spot urine samples for each time window was significantly different from 24-h urine sample (control value) except for two different time windows that were collected at 1600-1959 and overnight (P<0.05; Figure 1). Mean of spot urine samples in a specific time window that was statistically different from 24-h urine sample were appeared with red dots, while those that were not statistically different appeared as green dots in Figure 1. However, mean of spot urine sample that was collected overnight had a wider range between upper decision limit (UDL) and lower decision limit (LDL) compared to the mean of spot urine samples in another time window.

Equivalence test of urine osmolality from spot urine sample was also performed at each time window to compare them with the urine osmolality from 24-h urine sample by using

100 mmol·kg^{-1} as a bound (Table 2). As a result, only the mean of spot urine sample that was obtained at 0600-1159, 1200-1559, 1600-1959, and overnight was identified as equivalent compared to 24-h urine sample (P<0.05). However, only the mean of spot urine osmolality obtained at 1600-1959 was equivalent when lower the bound to 60 mmol·kg^{-1}. Pairwise correlation analysis of urine osmolality showed a moderate relationship between the mean of spot urine sample at 1600-1959 and a 24-h urine sample (R^2=0.657; Figure 2). The threshold obtained from ROC curve for the mean of spot urine sample at 1600-1959 was 840 with 91.3% area under the curve (AUC) (sensitivity: 82.5%, specificity: 88.1%) that was very close to the cutoff of 800 mmol·kg^{-1} from 24-h urine sample (Table 3).

DISCUSSION

To identify the optimal time window for the mean of spot urine sample to estimate the hydration status, a current study performed several ways to test urine osmolality from the mean of spot urine sample to see if they were corresponding to the 24-h urine sample. Urine samples were obtained from free-living healthy children, and almost half of them were not meeting the AI of water from IOM guideline. First of all, two time window from the mean of spot urine sample was identified as non-significantly different from a 24-h urine sample, 1600-1959 and overnight. However, the range between UDL and LDL of overnight was the widest among all the time windows as overnight had significantly less sample size compared to other time windows which make the conclusion less certain. Secondly, the equivalence test between the mean of spot vs. 24-h urine sample for each time window showed that the mean of spot urine sample was equivalent at four different time windows. However, the mean difference and CI of the spot urine osmolality only at 1600-1959 fell below the bound when it was set at 60 mmol·kg^{-1}. In addition, pairwise correlation analysis also showed a moderate relationship of osmolality between the mean of spot urine sample obtained at 1600-1959 and a 24-h urine sample (R^2=0.657). Lastly, AUC of the mean of spot urine sample at 1600-1959 with 24-h urine osmolality cutoff from ROC curve analysis was 91.3% that represents “excellent” test. These statistical analyses identified that urine osmolality obtained from the mean of spot urine sample at 1600-1959 was the most valid time window in measuring hydration status that corresponds to the urine osmolality from 24-h urine sample in children population, and this result is consistent with the previous study that was examined against adults (Bottin et al., 2016).

Although underhydration is reported to be associated with various health issues (Popkin & Rosenberg, 2010), the practicality of hydration assessment in free-living population is still controversial as there are more physiological and habitual factors that need to be considered other than clinical biomarkers (Armstrong, Kavouras, Walsh, & Roberts, 2016; Perrier, Vergne, et al., 2013; Perrier, 2017). Hydration biomarkers are varied, and multiple biomarkers can be used together occasionally to assess the hydration status especially during exercise in combination with weight loss measurement and thirst perception (Cheuvront & Kenefick, 2016; Lee et al., 2017). Plasma osmolality would be a proper marker for the one-time measurement to acute changes during exercise when setting more than 2% body weight loss as standard (Cheuvront, Ely, Kenefick, & Sawka, 2010). Urine osmolality also has been suggested as proper urinary index that is valid and non-invasive for hydration assessment to ensure sufficient urine output to maintain renal health and arginine vasopressin (AVP) level (Perrier, Bottin, Vecchio, & Lemetais, 2017), and it can be used relatively easy to estimate the hydration status in large-sample studies (Baron, Courbebaisse, Lepicard, & Friedlander, 2015). Especially, 24-h urine osmolality represents the consumed fluid throughout the day, and its cutoff of 500 $mmol \cdot kg^{-1}$ has been suggested as a simple indicator to evaluate optimal hydration (Erica T. Perrier et al., 2015).

Hydration status fluctuates throughout the day, and it can be affected by the amount of fluid intake, physical activity, and other surrounding environmental factors (Perrier, 2017). One study examined water intake in 52 healthy adults and reported that their urine osmolality fluctuated throughout the study (Perrier et al., 2013). In this study, they divided their participants into two groups; low drinker (<1.2 L/day) and high drinker (2-4 L/day). Urine osmolality of two groups got reversed according to the amount of water they consumed, however, urine osmolality in both groups still fluctuated throughout the day regardless of their habitual water intake. Due to this reason, a spot urine sample has not been recommended for hydration assessment (Cheuvront et al., 2015), yet, it still may be a useful biomarker for its convenience and efficiency especially in large-sample studies when used properly (Bar-David et al., 2009; Kenney et al., 2015; Stookey et al., 2012).

In conclusion, late afternoon around 1600-1959 may be the optimal time window to evaluate the hydration status effectively even in children as an alternative to 24-h urine sample when 24-h urine collection is not available especially in a large-sample study.

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APPENDIX

Table 1. Basic Characteristics

	3 y (n=43)	4-8 y (n=282)	9-13 y (n=216)	Total (n=541)
Age (y)	3.0±0.0	5.9±1.4*	10.8±1.4*†	7.6±3.0
Weight (kg)	16.2±2.9	23.4±6.3*	45.2±15.6*†	31.4±15.6
Height (cm)	99±4	119±11*	149±12*†	129±20
BMI ($kg \cdot m^{-2}$)	16.1±2.0	16.3±2.2	19.9±5.0*†	17.7±4.0
UOsm ($mmol \cdot kg^{-1}$)	660±205	732±233	681±247†	706±239
Void Frequency	6.2±2.0	6.8±2.9*	5.7±2.5†	6.4±2.7
TFI (mL)	1,026±515	1,257±569*	1,712±761*†	1,422±692
PWI (mL)	588±641	648±457	951±649*†	760±564
SSB (mL)	136±240	205±281	346±389*†	258±335
TFI		**Percentage (%)**		
Meet	44	49	46	48
Do Not Meet	56	51	54	52
800 Cutoff				
<800 $mmol \cdot kg^{-1}$	77	59	66	63
≥800 $mmol \cdot kg^{-1}$	23	41	34	37

* Denotes the difference between 3 y vs. 4-8 y or 3 y vs. 9-13 y (*P*<0.05)

† Denotes the difference between 4-8 y vs. 9-13 y (*P*<0.05)

BMI, body mass index; UOsm, urine osmolality; TFI, total fluid intake; PWI, plain water intake; SSB, sugar-sweetened beverage

Table 2. Equivalence test of urine osmolality from the mean of spot urine samples at different time windows with 24-h urine sample by using different bounds

Time window (hours)	Mean absolute difference (spot vs. 24-h; UOsm)	N	95 % CI of mean (mmol·kg^{-1})		Equivalence Test	
			Lower limit	Upper limit	Bound (mmol·kg^{-1})	
					100	**60**
0600-1159	67	377	49	85	Y	N
1200-1559	55	463	39	70	Y	N
1600-1959	41	471	28	54	Y	Y
2000-2359	87	359	71	103	N	N
Overnight	50	72	10	89	Y	N
First Morning	88	415	74	102	N	N

UOsm, urine osmolality

Table 3. Receiver operating characteristics curve of the mean of spot urine osmolality vs. 24-h urine osmolality cutoff (800 mmol·kg^{-1})

	Cutoff	Threshold	AUC	Sensitivity	Specificity
Spot Sample (1600-1959)	800	840	91.3%	82.5%	88.1%

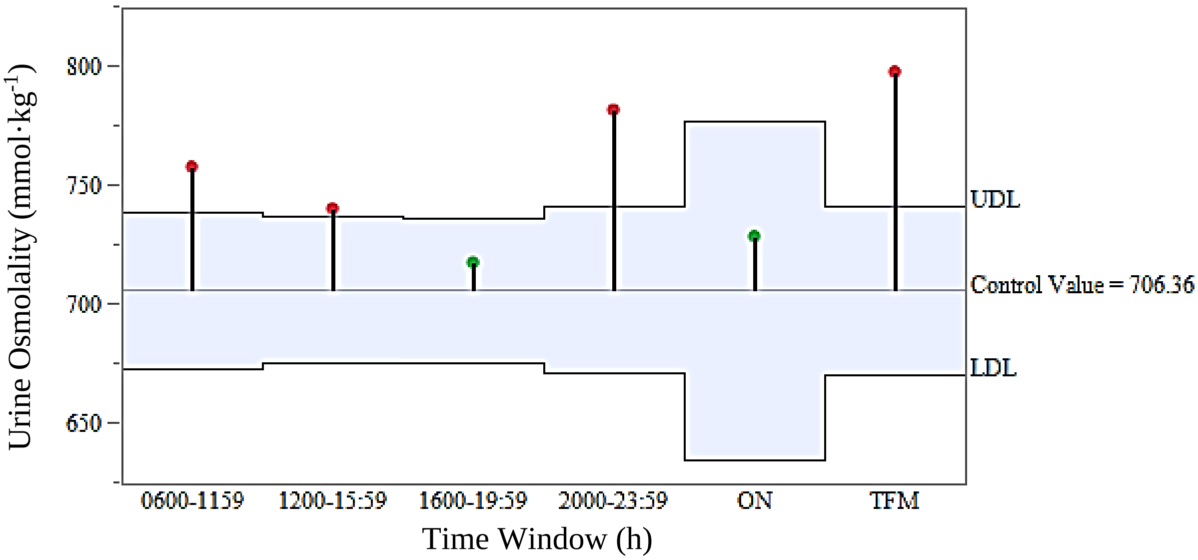
UOsm, urine osmolality

FIGURE LEGENDS

Figure 1. Mixed model multiple comparisons of the mean of spot urine sample with control decision chart
Control value = Mean value of 24-h urine osmolality
Red dot denotes the statistical difference from control value (P<0.05)
UDL, upper decision limit; LDL, lower decision limit; ON, overnight; FMS, first-morning sample

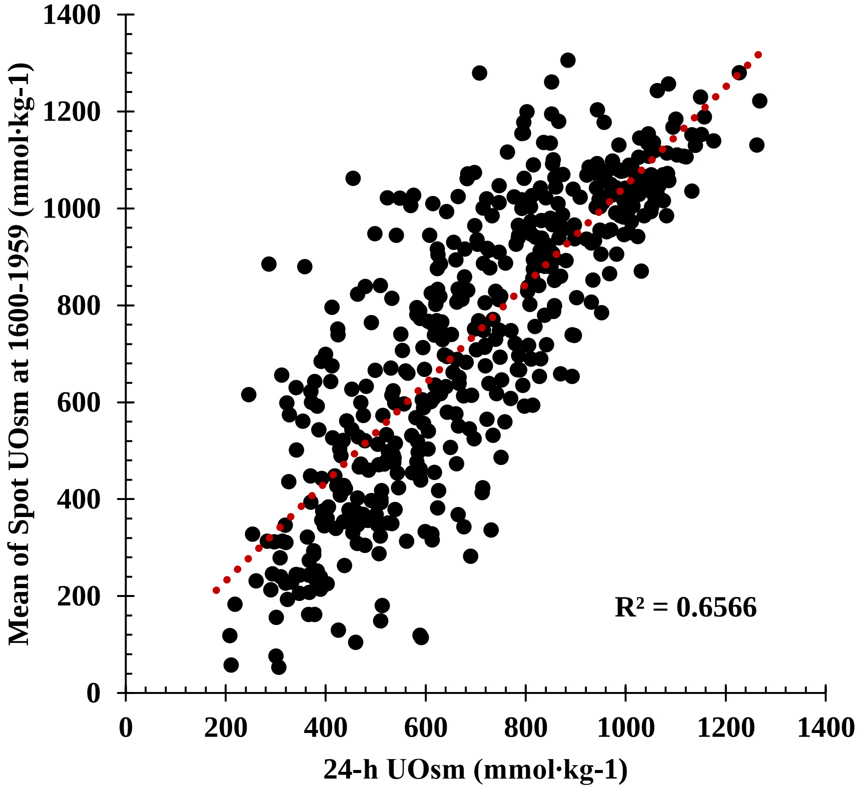
Figure 2. Correlation between the mean of spot urine osmolality at1600-1959 and 24-h urine osmolality

Figure 1.



Urine Osmolality (mmol·kg-1)
800
750
700
650
UDL
Control Value = 706.36
LDL
0600-1159
1200-15:59
1600-19:59
2000-23:59
ON
TFM
Time Window (h)

Figure 2.



1400
1200
1000
800
600
400
200
0
Mean of Spot UOsm at 1600-1959 (mmol·kg-1)
R² = 0.6566
0
200
400
600
800
1000
1200
1400
24-h UOsm (mmol·kg-1)

IV. CONCLUSION

These studies investigated the determinants of hydration in children. To examine this, we explored different factors that could influence children's water intake and hydration status. The factors were demographical (age), physiological (BMI), behavioral (water intake, SSB intake, SSB intake, and physical activity), and environmental factors. Environmental factors included parental influence such as the parent's marital status and education. For the assessment of children's hydration status, we performed the equivalence test between the mean of spot urine sample in six different time windows [morning (0600-1159), early afternoon (1200-1559), late afternoon (1600-1959), evening (2000-2359), overnight (2400-0559), and first morning (0600-0959)] with 24-h urine sample. Different statistical analyses were applied for the validity.

In Study 1, 59% of children appeared that they do not meet the water intake guideline by IOM and it was influenced by the parent's water intake. Children's water & fluid intake was affected by demographical (age), physiological (BMI), behavioral (SSB intake and physical activity), and environmental (parent's BMI, marital status, and education) factors. Lastly, the most contributing factors to children's water intake were age, BMI, and total protein intake, while the parent's marital status was the most contributing factor to children's hydration status.

In Study 2, mean of spot urine osmolality around 4 pm to 8 pm was equivalent to 24-h urine osmolality in children as the absolute mean difference and the confidence interval of this time window fell within bound of 100 $mmol \cdot kg^{-1}$. Mean of spot urine osmolality at this time window also had a moderate correlation (R^2=0.657) with 24-h urine osmolality and the AUC from ROC curve appeared as "excellent" (91.3%). Lastly, only the mean of spot urine osmolality at this time window was not significantly different with mixed model multiple comparisons.

These data suggest that there are various factors that influence the hydration in children, and it can be better determined when examined both water intake and hydration status together. We demonstrated that these the contribution of factors to children's hydration are different and they are influenced by their parents. Moreover, we identified the proper time window to measure hydration status in children for its convenience by comparing it with 24-h urine biomarker. There may be additional variables that need to be considered in our analyses, but this study provides support for the plausible factors that need to be considered in examining water intake and hydration status in children for future studies.

APPENDIX

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

March 22, 2017

MEMORANDUM

TO:	Stavros Kavouras Hyun-Guh Suh Zachary Lewis Bryce Wall Alexandria Aldridge Cameron Sprong Rachel Claunts Harrison Woods	Lisa Jansen Audrey Smith Zoe McKinney Alison Schoeder Katherine Montgomery Arlette Abigail Askar Mackenzie Ray LynnDee Summers	J.D. Adams Jordan Smith Adam Seal Cody Shopper Kyle Cook Reid Fawcett Dylan Scott
FROM:	Ro Windwalker IRB Coordinator		
RE:	PROJECT CONTINUATION		
IRB Protocol #:	16-02-574		
Protocol Title:	*HYBISKUS: Reference Values and Determinants of Hydration in Children 3-13 y*		
Review Type:	☐ EXEMPT ☒ EXPEDITED ☐ FULL IRB		
Previous Approval Period:	Start Date: 04/04/2016 Expiration Date: 04/03/2017		
New Expiration Date:	04/03/2018		

Your request to extend the referenced protocol has been approved by the IRB. If at the end of this period you wish to continue the project, you must submit a request using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. Failure to obtain approval for a continuation on or prior to this new expiration date will result in termination of the protocol and you will be required to submit a new protocol to the IRB before continuing the project. Data collected past the protocol expiration date may need to be eliminated from the dataset should you wish to publish. Only data collected under a currently approved protocol can be certified by the IRB for any purpose.

This protocol has been approved for 2,000 total participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or irb@uark.edu.

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