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The Contribution of Selected Urinary Solutes to the Determination of Urinary Osmolality in Guatemalan Preschool Children Consuming a Common Menu Offering

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Abstract

Background: Although water is the most abundant and most vital of all human nutrients, hydration is among the most ignored aspects of human nutrition. Many different solutes are eliminated by the kidneys in the urine flow, potentially contributing to the osmotic charge of this body fluid and acting as determinants of the urinary osmolality [Uosm].

Objectives: To measure urine osmolality concurrently with urea, uric acid, calcium, magnesium, potassium, sodium and inorganic phosphorus in a 24 h sample and to determine the patterns of their mutual interactions towards assessing the primary determinants of Uosm.

Methods: Seventy-eight children from 2 to 7 years old, 40 girls and 38 boys, with median age of 57 mo underwent 24 h urine collections, with an aliquot separated for measuring urine osmolality by freezing-point-depression osmometry and solute concentrations by various analytical chemistry techniques. Spearman correlations and multiple regression models were run to assess interactions.

Results: Backward-elimination multiple-regression models found that the urinary concentrations of inorganic phosphorus, urea, sodium, potassium and magnesium explained 95.1% of the variance in Uosm among the seven analytes quantified; calcium and uric acid made negligible contribution.

Conclusion: The analyses allowed us to confirm the determinant roles of urea and the principal electrolytes, sodium and potassium, for urine osmolality and to appreciate coordination in the collateral collinear associations with other excreted solutes.

Keywords: Urinary osmolality; Hydration; Electrolytes; Preschool children; Guatemala

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Introduction

Although water is the most abundant and most vital of all human nutrients [1,2], hydration is among the most ignored aspects of human nutrition. This is especially true for juvenile populations, for which there are limited data based on quantitative urine collections [3]. Interestingly, in a sample of preschool-aged children attending government-sponsored day-care centers in the western highlands of Guatemala, the urinary osmolality [Uosm] was among the lowest of those reported across the pediatric literature [3], signifying a relatively superior hydration status.

The volume of water from all sources, including beverages, water in recipes, intrinsic food moisture and metabolic water from energy-substrate oxidation, is one determinant of hydration

status [4]. The minute-by-minute retention or excretion of this water is governed, in part, by renal regulatory mechanisms that filter the circulating stream of plasma containing a host of small, electrically-charged and neutral uncharged constituents to be excreted in the urine. These atomic and molecular species, themselves, initially originate in the diet. In some instances, they can be turned over and excreted on the same day of ingestion, as in the case of sodium [Na], potassium [K] and chloride. Alternatively, they may have been incorporated for various time intervals in bone or muscle, as with calcium [Ca], magnesium [Mg] and phosphorus [Pi], or in any soft tissue such as muscle or visceral organs, as with the amino acid-derived nitrogen, excreted as urea. Furthermore, the nucleic acids in the plant and animal cells in the diet and from endogenous sources of human tissues yield uric acid, eliminated by the kidneys in the urine flow. As ions and minute molecules in the urine, these− and many other solutes−contribute to the osmotic potential of this body fluid and constitute the basis for the Uosm. For young children then, the daily water balance plus the dietary food selection and the turnover of growing tissues would interact, in combination, to determine the osmolality as measured in urine.

What the resultant variation in the excretion of these solutes and the influence on Uosm would be in situations where children had similar physical activity routines and common dietary offering is not known. In the present study, the 24 h urine specimens were collected from the aforementioned convenience sample of Guatemalan preschool children and a select group of two organic compounds (urea and uric acid) and four cationic electrolytes (Ca, Mg, K and Na) and one inorganic anionic compound (Pi) were measured. Uosm was determined by freezing-point depression osmometry, a putative indicator of human hydration status [5]. We present here the patterns of mutual interactions, and multiple regressions to assess the primary determinants of osmolality.

Subjects and Methods

Settings

The geographical setting, day-care center sites and the routines and menu offerings at the centers have been presented previously [3]. The study was conducted in the Western Highlands of Guatemala in the province of Quetzaltenango, located 220 km from Guatemala City and 2357 m above the sea level, with a land area of 1,943 km^2 and 24 municipalities [6]; the Secretariat for Beneficial Works of the First Lady [SOSEP] named three different daycare centers to our study, one located in La Esperanza, a semi-urban location (Center A); another located really close to the city, La Puerta del Llano, but classified as marginal-urban, (Center B); and the last one, and in the most rural setting, San Martín Sacatepéquez, (Center C). The centers share an 8 weeks menu cycle in common, with a 2-meal plus 2-snack daily offering.

Subjects

Seventy-eight children from 2 to 7 years old attending daycare centers from the SOSEP system in three areas of Quetzaltenango, Guatemala were enrolled in the study. The majority of them of them were of Mayan indigenous ascent; however, certain cultural behaviors, pastimes and physical characteristics varied among centers.

Exclusion criteria

Children were excluded if they did not attend one of the 3 selected daycare centers at least 80% of the center`s working-days during the 8 weeks within the study period. Also excluded were children whose parents refused to adhere to the urine collection routine, to participate in the study, or whose parents refused to sign the consent form were excluded. Eligible subjects were apparently healthy and with no restrictions in consuming the foods and beverages offered within the SOSEP menu.

Ethical considerations

Study protocol obtained ethical approval from The Center for the Studies of Sensory Impairment, Aging, and Metabolism's Human Subjects Committee in Guatemala City. Participants were required to have a consent form signed by a parent or legal guardian. Previous authorization was obtained from the director of SOSEP for the Quetzaltenango area. When the situation required, the investigation purchase the prescribed items to assure the complete offering of the assigned SOSEP menu. A physician responded to the findings of the diagnostic tests (e.g., hemogram, stool and urine tests), and delivered deworming treatment along with medical prescription, e.g., for oral iron supplementation, or medical care, e.g., antibiotics for urinary tract infection.

Urine collection, aliquoting and storage

After two previous collections, third sample of 24 h urine was obtained in plastic 24 h collection container (BD Vacutainer® No.364999 Becton-Dickinson Co., NJ, USA). Urine collection was started in the daycare center at the time each child arrived. Collection in daycare centers was supervised by investigators and SOSEP personnel and continued at home with the parents' assistance until it was finished, 24 h thereafter. When collection became incomplete either at daycare center or home, it was restarted the following day.

Three rounds of 24 h urine collection were conducted in Quetzaltenango, Guatemala between August and November 2012. During the third one, samples were well mixed, urine volume was determined, and two additional 4 mL aliquots were stored at -80°C in Guatemala City. One of those was shipped to Granada, Spain on dry ice and stored at -80°C until analyses, total storage time from 43 to 52 weeks.

Measurement of urinary osmolality

After volume measurement with volumetric cylinders and 4 mL aliquots storage at -80°C in ultra-cold freezers, the samples were mixed for about 3 min to assure homogeneity. A 50 µL aliquot of urine was pipetted into the sampling chamber of an Osmomat 030 osmometer (Gonotec, Berlin, Germany) and the Uosm was read out in mOsm/kg of urine. The technique had a measurement precision with a coefficient of variation of 1%.

Measurement of urinary analytes

The series of seven constituents were measured across different analytical laboratories of Scientific Instrumentation Center (CIC) of the Center of Biomedical Research, University of Granada). The CIC bio-analysis unit ran the tests to determine urea, and uric acid using the automatic biochemical analyzer BS200 (Mindray, Shenzhen, China). Analysis of Na, K, Ca and Mg by atomic emission spectroscopy with inductive-coupled plasma (ICP-AES) using the model Optima 8300 (PerkinElmer, Waltham, MA, USA) in the structures analysis and determination unit. Pi was measured using the Fiske-Subbarow, direct method, kit (catalog #997609, QCA, S.A., Spain).

Data handling and statistical analysis

All descriptive and analytical statistics were calculated using SPSS version 20 (IBM, Chicago, IL, USA). Normality in the data distribution was determined using the Kolmogorov-Smirnov test. Descriptive statistics are presented as mean ± SD, median, 95% confidence interval and range for 24 h total production and concentration for each solute and for urine volume and osmolality. Spearman correlation coefficient was determined for all variables, and a multiple regression backwards step analysis was run in order to predict values of urine osmolality from the urine solutes measured for this study. A probability level of <0.05 was accepted as the criterion for statistical significance.

Results

Characteristic of the subjects

There were a total of 78 subjects, 40 girls and 38 boys, whose urinary data were complete for the at least one of variables. Median age was 57 mo with mean of 57 ± 15 and a range from 23 to 81 mo. Disaggregated characteristics by sex and site are shown in **Figure 1**.

Descriptive statistics of urine volume, Uosm and selected solutes

The descriptive statistics of median, 95% confident intervals and maximal and minimal values for urine volume and Uosm along with the solutes of interest are presented in **Table 1**. The solutes

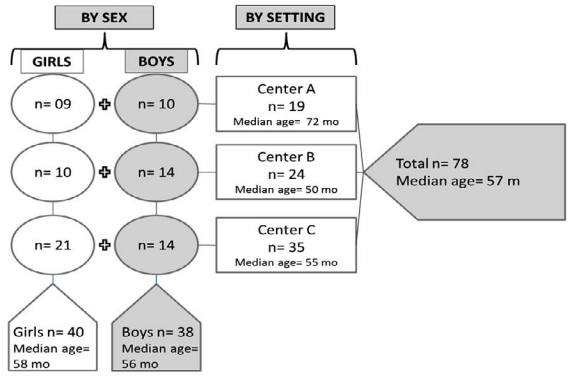

Figure 1 Demographic characteristics of the sample.

Table 1: Descriptive statistics of Urine volume, Usom and concentrations and daily excretions selected analytes (Uosm: Urine osmolality; Na: Sodium; K: Potassium; Ca: Calcium; Mg: Magnesium; Pi: Inorganic phosphorous).

Variable (unit)	Median	95% CI	min–max
Urine volume (mL/d)	460	436-575	65-1670
Uosm (mosm/kg)	430	407-491	115-1102
Solute concentration			
Uric acid (mg/dL)	15.5	14.8-16.5	6.8-26.8
Urea (mg/dL)	100.0	76.3-117.2	0.02-283.0
Na (mg/dL)	183.0	172.2-210.3	19.3-441.6
K (mg/dL)	114.2	118.5-141.1	68.5-325.6
Ca (mg/dL)	6.3	6.2-8.7	0.6-34.4
Mg (mg/dL)	5.7	5.5-7.2	0.7-15.2
Pi (mg/dL)	21.9	23.6-31.9	2.35-75.15
Daily excretion of solute			
Uric acid (mg/d)	69	68-90	9-231
Urea (mg/d)	466	392-660	0.1-1914
Na (mg/d)	799	772-1040	58-2430
K (mg/d)	567	531-688	90-1500
Ca (mg/d)	27	29-46	3-206
Mg (mg/d)	26	25-35	1.4-94
Pi (mg/d)	111	105-145	5-411

are described both as concentration (units/dL) in the middle panel and as amount per full collection (units/d) in the lower panel. With the exception of Na concentrations, all concentration and daily-output values for the solutes did not have a normal, Gaussian distribution.

Associations among the variables

As seen in the cross-variable Spearman hemi-matrix **(Table 2)**, a total of 22 correlation coefficients (61%) met the 5% probability criterion when the total number of subjects having both paired variables is included. Of these 13 were direct and 9 were inverse. When only the 64 children with all variable reported are analyzed, this declines to 19 (53%, 12 direct and 7 inverse) (data not shown). Of the statistical significant associations, r values ranged from -0.260 (urinary volume with Na, P = 0.022) to 0.836 (Uosm with Na, $P<10^{-3}$).

Multiple regression model of prediction of Uosm

In order to determine the relative contribution of the various urinary variables to the resultant Uosm measured across these preschool children, we performed backward-elimination multiple regression in conjunction with ANOVA **(Table 3)**. With all 9 variables as the standard of explaining 100% of the measurable balance, the r^2 variables remained stable, virtually no loss in explained variance for predicting Uosm down to the 5 analytes. The variables eliminated in penultimate and ultimate steps of backward elimination were Ca and uric acid, respectively. The 5/ five analytes remaining in the final round Pi, K, Na and Mg, all expressed as concentration in mg/dL; these explained 95.1% of the variance in Uosm.

Table 2: Spearman correlation hemi-matrix of Uvol, Uosm and 7 selected urinary analytes concentrations for 78 preschool children. (Uvol=urine volume; Uosm=urine osmolality; Na=sodium; K=potassium; Ca=calcium; Mg=magnesium; Pi= inorganic phosphorous; *Significant at a 0.05 level; **Significant at a 0.01 level)

Variable		Uvol (ml/24 h)	Uosm (mOsm/kg)	Urinary Uric Acid (mg/dL)	Urinary Urea (mg/dL)	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Pi (mg/dL)
	r-value	1.000								
Uvol	P-value									
(ml/24 h)	n	78								
	r-value	-0.363**	1.000							
Uosm	P-value	0.001								
(mOsm/Kg)	n	78	78							
	r-value	0.090	0.024	1.000						
Urinary Uric	P-value	0.436	0.833							
Acid (mg/dl)	n	78	78	78						
	r-value	-0.079	-0.742**	0.131	1.000					
Urinary Urea	P-value	0.532	<0.001	0.299						
(mg/dl)	n	65	65	65	78					
	r-value	-0.260*	0.836**	0.138	-0.516**	1.000				
Na (mg/L)	P-value	0.022	<0.001	0.23	<0.001					
	n	77	77	77	64	78				
	r-value	-0.312**	0.580**	0.051	-0.297*	0.544**	1.000			
K (mg/L)	P-value	0.006	<0.001	0.658	0.017	<0.001				
	n	77	77	77	64	77	78			
	r-value	0.003	0.289*	0.207	-0.330**	0.334**	-0.063	1.000		
Ca (mg/L)	P-value	0.981	0.011	0.07	0.008	0.003	0.584			
	n	77	77	77	64	77	77	78		
	r-value	-0.178	0.771**	0.178	-0.577**	0.629**	0.321**	1.407**	1.000	
Mg (mg/L)	P-value	0.122	<0.001	0.121	<0.001	<0.001	0.004	<0.001		
	n	77	77	77	64	77	77	77	78	
	r-value	-0.173	0.785**	-0.038	-0.651**	0.524**	0.459**	0.138	0.695**	1.000
Pi (mg/dL)	P-value	0.13	<0.001	0.743	<0.001	<0.001	<0.001	0.232	0.000	
	n	78	78	78	65	77	77	77	77	78

Table 3: Coefficients of the third and final backward elimination multiple regression model for the dependent variable urine osmolality, presenting the urine solutes measured in the present study (uric acid, urea, Na, K, Ca, Mg, Pi) as independent variables (Uosm: Urine osmolality; Na: Sodium; K: Potassium; Mg: Magnesium; Pi: Inorganic phosphorus; *Uric acid and Calcium were eliminated in the first two rounds of modeling; **R^2=0.951; R=0.975 (n=64).

		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
No.	Model	B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
				*Dependent variable: Uosm***				
3	(Constant)	142.810	18.649	-	7.658	0.0001	105.481	180.139
	Urea	-0.369	0.057	-0.252	-6.444	0.0001	-0.484	-0.254
	Na	0.095	0.007	0.538	13.525	0.0001	0.081	0.109
	K	0.047	0.010	0.155	4.666	0.0001	0.027	0.068
	Mg	0.535	0.157	0.140	3.407	0.001	0.220	0.849
	Pi	1.409	0.364	0.156	3.865	0.0001	0.679	2.138

Discussion

In the assessment of human hydration, we cannot rely on the osmolality of the plasma in the circulation, itself [7], as it is tightly and homeostatically regulated within a narrow band of 280-295 mOsm/kg of plasma [8,9], just as is the blood pH and serum Na and K concentrations, to avoid a collapse of vital physiological function. Moreover, it is common in clinical practice to make a surrogate estimate for plasma osmolality using a formula including the circulating concentrations of Na, K, glucose and urea nitrogen, which comes within about 10 mOsm/kg of the true value as measured by osmometry [8].

We can get some indication of hydration, however, by assessing the total urinary volume and indicators of the resultant systemic regulation in the osmolality in the excreted urine (Uosm), which is constituted by the organic and inorganic solutes dissolved in that volume of urine [5,7]. Manz and Wentz, 2003 [5] introduced the term "euhydration" to define the range of hydration state, defined by Uosm, consistent with normal function. Within that

band, the Guatemalan preschoolers in this series have a relatively low Uosm in a comparative perspective [3].

Since the children came from similar settings and had a common menu offering, it is notable how widely ranging were the variables of urinary concentration. This begins with the variation in urine volumes on the day of collection. This is determined not only by water intake, but also by the drives for excretion across the various routes. It should be remembered, moreover, that a common menu means cumulatively consistent offerings of food and beverages over a 40-day cycle, but on any individual collection day, the meals could be quite distinct from center to center. With that would follow different intakes of salt, protein and the diet's contribution of the minerals of interest.

The SOSEP diet and subjects' body size and composition are two major factors in the excretion of solutes in urine. It is useful to try to place the descriptive statistics on 24 h excretion of selected solutes in a context of publication on for daily urinary outputs in child populations from the comparative literature. The daily excretion values from **Table 1** are expressed as medians, as none met criterion for Gaussian normality; for the purposes of comparative discussion, however, they have been converted to arithmetic means, the format used in all of the cited publications. With respect to the organic solutes, urinary urea is considered to reflect recent dietary protein intake; the mean of 525 mg/24 h is considerably lower than the ~3 g reported in 11 Japanese children below 4 years of age [10]. Uric acid is derived from the turnover of nucleic acids from both the exogenous diet and the turnover of endogenous tissues. Our mean uric acid value of 79 mg/24 h is about half that of the 175 mg from 44 Brazilian preschoolers [11].

Our median full-collection Na was approximately 0.9 g, which would correspond to 2 g of salt (sodium chloride). This compares to 1.2 g in the same 11 Japanese preschoolers cited above [10]. Both of these daily urinary outputs were greatly surpassed by 6 year-olds in Finland, with a 1.6 g average [12]. The corresponding K excretions in the three series are equally aligned with a respective: 0.61 g in Western Guatemala; 0.86 g in Japan; and 1.25 g in Finland. The lower Na intake for Guatemala is compatible with observations made with lithium-labeled salt by Melsa-Boonstra et al. [13], who documented a 1.8 ± 0.6 g salt/d in rural boys aged 6 to 12 y.

Urinary Ca [11,13] and Mg [14] outputs in these citation was expressed as mg per kg body weight of the subject per 24 h. For comparative ends, then, we have used a median weight of 14.6 kg for our sample of preschoolers to unify the excretion units to adjust an arithmetic mean of 37.8 mg of Ca in full collections. This produces values of 2.60 mg/kg/d for Guatemalan preschoolers, which compared to 1.44 mg/kg/d for 125 Brazilian children in the 2-18 years range [11], and 2.38 mg/kg/d for 52 healthy London children aged 1-15 years [14]. For Mg, our similarly adjusted mean urinary value of 2.0 mg/kg/d contrasts with the higher 2.82 mg/kg/d in 23 of the London children [14]. For all of these selected solute excretions compared, the failure to get complete collection in about half of the instances predisposes to a greater or lesser degree of underestimation for our series.

The primary task of our analysis for solute interactions was to tabulate association. With a total of 36 cross-variable correlations, up to two associations could be expected to be significant by chance alone. Thus, at least 20 of the correlation coefficients meeting the <0.05 criterion are likely to be truly significant. Furthermore, the pattern of associations was interesting. Three variables, Uosm, Na and K, showed significant cross-correlations in 7 of 8 instances 87.5%); in each case it was uric acid concentration that failed to associate at a significant level. Thereafter, in descending order, significant correlations were shown for Mg in 6 instances, Pi in 5, Ca in 4, and urinary volume in 3. Uric acid was the only solute of interest that showed no mutual associations whatsoever.

Of the nine significant inverse associations seen, three involved urinary volume, and remainder were the entire series of significant relationships with urea concentration. The inverse volume relationships, with Uosm, Na and K are intuitively understandable, as a "dilution effect." i.e., more water lowers concentration of the electrolytes most associated with Uosm, and Uosm, itself. The inverse relationship with urea and osmolality, however, is not intuitively obvious. In fact, as stated, for prediction of plasma osmolality, urea is one of the positive determinants [8,9]. In order to verify the consistency of this indirect relationship between urea and Uosm, we generated Spearman partial correlations for seven subgroups **(Table 4)**. Consistent with the direction at the total-sample level, inverse and significant associations were seen for each sex, for the sample polarized for age, and within each of the three different centers. In fact, the inverse relationship of urinary urea and Uosm follows the classical basic physiology of renal regulation of water balance [15,16]. In order to conserve water within the body, and hence excrete more concentrated urine, urea is more aggressively absorbed in the counter-current exchange in the terminal inner medullary collecting duct (loop of Henle) of the kidney [17]. Hence, less urea in the urine is at the basis of the generation of higher osmolality and vice versa.

We recognize a series of strengths and limitations in the present study. A major strength is that it addresses the age-group of young children, a population segment relatively ignored in the existing literature. Furthermore, it brings together the skilled analysis with modern osmolality equipment with an effort at quantitative collection of urine and the concurrent analysis of seven important solutes in human urine derived from the diet, endogenous tissue breakdown or both. Our children are offered a common and controlled dietary menu of both foods and beverage within the institutional center-day, which might theoretically narrow the

Table 4: Full and partial Spearman correlation coefficients for the association of urea concentration (x-axis) and Uosm (y-axis) (Uosm: Urine osmolality).

Correlation	r-value	P-value
Total (n=65)	-0.742	<0.001
Girls (n=32)	-0.772	<0.001
Boys (n=33)	-0.658	<0.001
Older (n=35)	-0.842	<0.001
Younger (n=30)	-0.594	0.001
Site A (n=19)	-0.651	0.003
Site B (n=19)	-0.858	<0.001
Site C (n=27)	-0.767	<0.001

sample-wide variance. At the same time, the latter constitutes a contextual limitation, as that the study series is a convenience sample of children with unique dietary long-term homogeneity, generalization to the population at large would not be justified. Furthermore, although the intention was to collect quantitative 24 h urine excretion on all collection occasions, an internal indicator [18] suggests that this was accomplished only in a slight majority of instances [3].

In conclusion, a situation in which a defined offering of an institutional menu and efforts at 24 h urinary collections converged in the study of systematic Uosm measurement; this has facilitated our gaining insights into the relationship of urinary excretion of selected solutes in the context of their relationship to Uosm, which is a proxy indicator for human hydration state. The most striking features overall in this group of relatively well-hydrated preschool children is the inter-individual variation in excretion of the specific species analyzed. The analyses allowed us to confirm the determinant roles in Uosm of urea and the principal electrolytes, Na and K and to appreciate a harmony in the collateral collinear associations with other excreted solutes.

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