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Evaluation of physiological measures for correcting variation in urinary output: Implications for assessing environmental chemical exposure in children

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Urinary contaminant concentrations are commonly adjusted by creatinine to account for the variability in urinary output. This approach may not be optimal among children due to developmental growth of muscle mass and the associated increase in creatinine formation. An alternative approach is to measure the specific gravity of the urine sample, which reflects the solute concentration of the urine. We compare the appropriateness of urinary creatinine and urinary-specific gravity as factors for correcting morning and evening spot urine samples collected from 23 children (3–11 years) for a total of 41 days in four different seasons. Two linear mixed-effects models were fit using age, sex, season, and sample collection time (morning/evening) as predictors with specific gravity and creatinine as dependent variables. Specific gravity was significantly associated with the sample collection time ($P<0.001$) with morning samples higher than evening samples. Creatinine was significantly associated with season ($P<0.05$), sample collection time ($P<0.0001$), and age ($P<0.0001$). Creatinine levels were higher during the summer compared to the other seasons, higher in the morning compared to the evening, and higher with increases in children's age. Normalizing the children's spot urine samples using creatinine would introduce bias to the data analysis. Whereas using specific gravity to correct for variable urinary output would be more robust. In addition, measuring specific gravity is relatively easy, does not require the use of chemicals, and the results are available instantaneously.
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Introduction

The goal of biomonitoring is to quantitatively assess environmental or occupational chemical exposure by measuring either the parent chemical or its metabolite in various specimen samples. Single spot urine samples, for example, are routinely collected from study participants to evaluate exposures to pesticides, heavy metals, and other contaminants (Barr and Angerer, 2006; Paustenbach and Galbraith, 2006; Angerer et al., 2007). The urinary concentration of a chemical or its metabolite is commonly expressed as mass per unit volume (μg/l). Urinary metabolite concentrations are governed by the quantity of a chemical present in the blood, the rate at which the chemical is excreted from the blood, and the amount of fluid excreted by the kidneys (Boeniger et al., 1993). Thus, changes in the amount of fluid excreted will result in variability in urinary metabolite concentrations, irrespective of exposure to the chemical. To account for this variability, the renal elimination rate, the rate at which the kidney filters the blood, needs to be measured. However, as this rate is not readily accessible, alternative measures that reflect urine concentration and that can easily be derived from the urine sample are often used to adjust the urinary concentration of chemicals. For a measure to be useful as a correction factor, it must not systematically vary across demographic groups of interest, such as age or sex, or across study factors, such as season or time of day. Otherwise, comparisons among individuals that differ across these factors may be artificially set apart, irrespective of actual exposure levels.

Urinary creatinine, a chemical by-product generated from muscle metabolism (Cocchetto et al., 1983), has been used to correct for urinary concentration because its production and excretion are relatively constant due to homeostatic control mechanisms, and creatinine excretion is mostly independent of urine flow (Boeniger et al., 1993). The underlying theory for creatinine correction is that the blood ratio of creatinine to the chemical of interest is maintained in urine because both compounds are filtered at the same rate by the kidney. Creatinine correction is relatively well accepted for adult

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samples, although controversy does exist due to the fact that both exogenous (e.g. diet, exercise) and endogenous factors (e.g. age, sex, muscle mass) can affect creatinine production (Boeniger et al., 1993). A recent report examining children's creatinine concentration as the children matured from preadolescence to adolescence clearly showed an increase in creatinine concentration with age (Ortiz-Perez et al., 2005). In addition, diurnal variations have been found with morning creatinine concentrations higher than evening creatinine concentrations (Barr et al., 2005). This finding is consistent with human physiology, as the time from the last void is extended relative to other voids during the day, and fluid consumption rarely occurs between evening and morning. Furthermore, seasonal variations in young children's (<6 years old) creatinine levels have been documented with higher creatinine levels in summer urine samples relative to fall urine samples (Freeman et al., 1995; O'Rourke et al., 2000). Overall, the documented variability in urinary creatinine concentration within and among persons, as well as across time, further highlights the need to evaluate the applicability of correcting urinary concentrations of chemicals by creatinine levels in children.

Specific gravity is another measure that can be used to adjust for urinary output. Specific gravity is the ratio of densities between the targeted aqueous solution and pure water, and is determined by both the number and size of particles in the liquid. Urinary-specific gravity is a valid indicator of urinary osmolality, the gold standard measurement of soluble particle concentrations in the urine (Chadha et al., 2001). Although urinary-specific gravity is not as commonly used as creatinine for correcting the variations of urine output, several studies have shown that specific gravity corrections introduced less variability to data sets than creatinine corrections (Haddow et al., 1994; Ikeda et al., 2003; Meeker et al., 2004; Miller et al., 2004). In addition, there is little evidence suggesting urinary-specific gravity may vary with demographic or temporal factors, as we only found two studies linking urinary-specific gravity with age (Freeman et al., 1995; Moriguchi et al., 2005), and no studies reporting a temporal pattern in urinary-specific gravity (Polat et al., 2006). Specific gravity-adjusted urinary metabolite concentration is calculated using the following equation (Newman et al., 2000):

$$\text{Chemical Concentration}_{\text{SG corrected}} = \frac{\text{Urinary chemical concentration}}{(\text{specific gravity} - 1) \times 100}$$

Specific gravity is influenced by urinary flow rate. The above specific gravity correction is based on the assumption that the ratio of the metabolite and total dissolved solids remains constant as urinary flow fluctuates throughout the day. The extent that this ratio changes with urinary flow represents the error that may be introduced by this simplistic correction approach. A modified SG correction has been proposed that eliminates the need for this assumption; however, it requires the estimation of the affect of urinary flow on the excretion rate of the metabolite (Vij and Howell, 1998).

In the present report, we compare the appropriateness of urinary creatinine and urinary-specific gravity as potential correction factors for children's urinary output using spot urine samples collected in the Children's Pesticide and Exposure Study in Seattle, Washington (CPES-WA). We examine the effects of age, sex, season, and sample collection time on children's urinary-specific gravity and creatinine concentration.

Methods

The CPES-WA was conducted in Seattle, Washington from July 2003 to May 2004. Details of the study design and methods have previously been published (Lu et al., 2006a, b). A total of 23 children (10 girls) aged 3–11 years were recruited from local schools for a 15-consecutive-day sampling period in summer 2003, with repeated samplings of 12 consecutive days in fall 2003, and of 7 consecutive days in both winter and spring 2004. Eligible children were those exclusively consuming conventional diets, spending most of their time in one residence, and willing to follow the study protocol. The total number of the original 23 participating children completing each of the sampling periods was 23 for the summer, 21 for the fall, 20 for the winter, and 19 for the spring. Written consent or oral assent was obtained from parents and older children, or from younger children, respectively. The University of Washington Human Subject Division (no. 03-5899) approved the use of human subjects in this study. The Emory University Internal Review Board (no. 084-2005) approved continued analysis of this data set. This report only includes measures of urinary creatinine concentrations and urinary-specific gravity across the study year and does not include any pesticide exposure results.

Urine Sample Collection and Analysis

During each study day, the first morning void and last void of the day (before bedtime) were collected from each child. Participants were instructed to collect the full void and then to immediately refrigerate the sample or place it in a cooler with ice packs. Each morning, the voids from that morning and the night before were transported to the laboratory on ice by study personnel. Upon arrival to the laboratory, the total volume and specific gravity of the urine were measured. Specific gravity measurements were made with a portable, handheld refractometer (VWR International, West Chester, PA, USA); this refractometer has a urinary-specific gravity range from 1.000 to 1.05, 0.001 graduated intervals, and values marked at each 0.01

graduation. The urine samples were then aliquoted and stored at −20°C until creatinine analysis was performed in the National Center for Environmental Health at the Centers for Disease Control and Prevention in Atlanta, GA, USA. Creatinine was measured photometrically using kinetic colorimetric assay technology with a Hitachi 911 automated chemistry analyzer (Roche Diagnostics, Indianapolis, IN, USA). A total of 1633 first and last daily urine voids from the CPES-WA children across the four seasons were analyzed.

Statistical Analysis

Plots confirmed a normal distribution for creatinine and specific gravity; as such, the descriptive statistics include the mean and standard deviation. For comparison, both creatinine and specific gravity were evaluated within the widely adopted guidelines for valid urine samples set forth by the World Health Organization (WHO) (WHO, 1996) and the US Department of Health and Human Services (DHHS, 1999); however, it should be noted that these guidelines were established for adult urine samples. Comparisons were also made with the Third National Health and Nutrition Examination Survey (NHANES III) and other reported measures of urinary creatinine and specific gravity from same-aged children. The influence of age, sex, sample collection time, and season on creatinine and specific gravity was investigated using two separate linear mixed-effects models with compound symmetry covariance structures and random intercepts for each participant. This approach accounts for the repeated measures and variation in baseline among participants. Sex, season, and sample collection time were defined as nominal variables in theses models; and age, a continuous variable, was entered as a covariate. Note that age was defined as the child's age at the start of the study. Statistical significance was defined to be a *P*-value less than 5%.

Results

Descriptive statistics of urinary creatinine concentrations grouped by sex, collection time, and season are listed in Table 1. Overall, the mean urinary creatinine concentration was 88.62 mg per 100 ml, which falls below the confidence interval for the mean creatinine concentration in the NHANES III data of children age 6–11 (Barr et al., 2005), but does fall into the creatinine range reported by Freeman et al. (1995) that includes children less than 6 years of age. Six percent of the 1633 ($N = 105$) urine samples had urinary creatinine concentrations below the WHO guideline (<30 mg per 100 ml). No measurement exceeded the WHO limit of 300 mg per 100 ml. This pattern of relatively dilute urine from children has been similarly reported in other studies (Freeman et al., 1995; O'Rourke et al., 2000; Barr et al., 2005).

Table 1 also provides the descriptive statistics for urinary-specific gravity by sex, sample collection time, and season. Overall, the mean urinary-specific gravity was 1.021, which was slightly higher than the children's specific gravity means reported by Freeman et al. (1995) and similar to those reported in same-aged children by Polat et al. (2006). Only five of the children's urine samples were below the DHHS (1999) acceptable range for urine specimens, which is defined as a urine sample with both a creatinine value less than 20 mg per 100 ml and a specific gravity value less than 1.003.

Results from the linear mixed-effects model (Table 2) demonstrated that age, sample collection time, and season are significantly associated with creatinine levels in the urine. As seen in Figure 1, the dependency of urinary creatinine levels on children's ages is visible. A seasonal increase in urinary creatinine levels was observed during the summer as well as a diurnal variation of increased urinary creatinine levels in the morning. The association between sex and

Table 1. Descriptive statistics of urinary creatinine concentrations and specific gravity measured in the CPES-WA children over a 12-month period.

	N^a	Creatinine (mg/100 ml)				Specific gravity			
		Mean (SD)	Min	Median	Max	Mean (SD)	Min	Median	Max
Sex									
Male	969	84.9 (41.7)	3.2	80.5	276.9	1.0200 (0.0064)	1.000	1.021	1.034
Female	664	94.2 (42.7)	4.5	91.5	285.1	1.0216 (0.0063)	1.001	1.023	1.041
Sample collection time									
First morning void	828	98.5 (41.2)	3.2	95.2	285.1	1.0214 (0.0057)	1.000	1.022	1.041
Bedtime	805	78.5 (41.1)	5.2	73.3	220.2	1.0199 (0.0070)	1.001	1.021	1.035
Season									
Summer (15 consecutive days)	646	91.8 (43.9)	3.2	87.7	225.0	1.0205 (0.0066)	1.000	1.021	1.041
Fall (12 consecutive days)	469	85.8 (40.8)	10.2	81.1	276.9	1.0208 (0.0062)	1.004	1.021	1.034
Winter (7 consecutive days)	267	85.7 (39.1)	7.4	83.1	243.4	1.0210 (0.0061)	1.004	1.022	1.034
Spring (7 consecutive days)	251	88.9 (44.1)	5.2	84.7	285.1	1.0206 (0.0067)	1.002	1.022	1.032

[a]Two urine samples were missing specific gravity measures.

Table 2. Results of the linear mixed effect models examining the association between age, sex, sample collection time, and season with creatinine and specific gravity measured from children's urine samples collected for a total of 41 days in four different seasons.

	Creatinine		Specific gravity	
Source	Coefficients	*F*-value (Pr > *F*)	Coefficients	*F*-value (Pr > *F*)
Intercept	44.64	20.6 (<0.001)	1.02	253312 (<0.001)
Age	5.68	14.4 (<0.001)	−0.0002	0.312 (0.583)
Sex		4.2 (0.055)		2.03 (0.17)
Male	−15.44		−0.0020	
Female	0[a]		0[a]	
Collection time		131.2 (<0.001)		24.1 (<0.001)
First morning	19.65		0.0014	
Bedtime	0[a]		0[a]	
Season		3.4 (0.018)		1.3 (0.28)
Spring	2.08		−0.0006	
Summer	6.81		−0.0007	
Fall	1.52		−0.0003	
Winter	0[a]		0[a]	

[a]Coefficient set to zero because it is redundant.

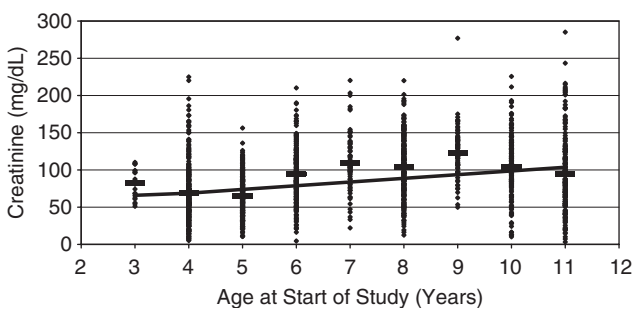

Figure 1. The correlation line between urinary creatinine concentration and children's age is derived from the fixed effect model (Table 2) with all other covariates set to their averages. Dashes indicate the mean creatinine concentration at each age.

creatinine approached significance, with female children tending to have higher creatinine levels than male children. When this model was rerun omitting the participant with the lowest (male, age 5 years, 47.99 μg per 100 ml) and highest (female, age 11 years, 128.72 μg per 100 ml) mean urinary creatinine concentrations, the marginal effect of sex was no longer present (all other main effects remained significant).

Results from the linear mixed-effects model investigating specific gravity were different to those for creatinine. Neither age, nor sex, nor season was significantly associated with urinary-specific gravity levels. Figure 2 shows the independence of specific gravity and children's age. However, like creatinine, a diurnal variation was found, with specific gravity higher in the morning samples (Table 2). Notably, the relationship between specific gravity and creatinine is not linear (Figure 3). The primary differences between the two measures appear in the upper levels of creatinine, where specific gravity does not increase despite the higher creatinine values.

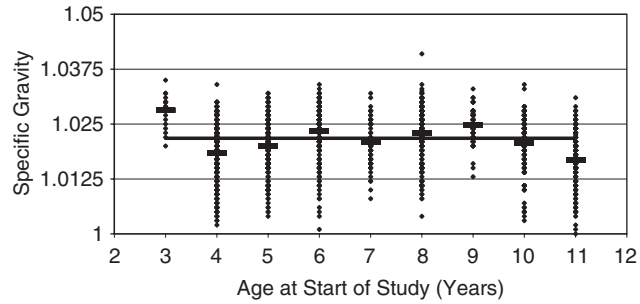

Figure 2. The correlation line between urinary-specific gravity and children's age is derived from the fixed effect model (Table 2) with all other covariates set to their averages. Dashes indicate the mean specific gravity at each age.

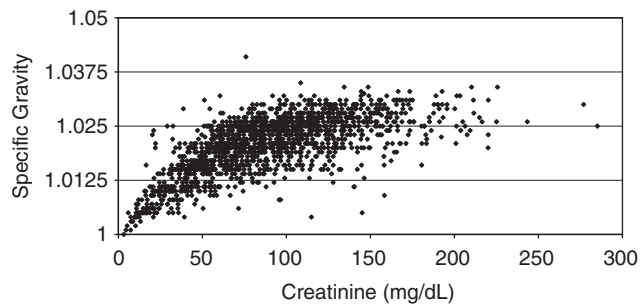

Figure 3. The relationship between urinary-specific gravity and urinary creatinine concentration is non-linear: specific gravity does not increase with the upper values of creatinine.

Discussion

Concerns have been raised as to the reliability of using creatinine to adjust for variable urine concentrations in children (O'Rourke et al., 2000). This concern stems from the fact that muscle mass is the primary predictor of creatinine production (Cocchetto et al., 1983) and increasing age during childhood parallels the developmental growth of muscle mass (Trowbridge et al., 1982). Confusion as to whether concentrations of chemicals in children's urine should be corrected using creatinine is reflected in the literature: some research groups have applied creatinine correction in biomonitoring work with children (Aprea et al., 2000; Curl et al., 2002), whereas others have reported the uncorrected concentration levels and used the children's urinary creatinine only to determine whether urine sample is

valid (Adgate et al., 2001; Lu et al., 2001; Bradman et al., 2007; Morgan et al., 2007). This issue is critical to risk assessment as an accurate measure of exposure is necessary for dose estimation and subsequent comparisons to benchmark doses, such as the acceptable daily intake published by the WHO (1997) or the reference dose established by the US EPA (2008).

The CPES-WA data set highlights the need to consider the influence of temporal variations on creatinine concentration. Creatinine concentration was highest during the summer season. This finding raises concerns regarding comparisons of creatinine-adjusted urinary chemical levels made across seasons either within or between study participants. For example, because seasonal variation in pesticide exposure is probably due to the documented seasonal differences found in dietary exposure (Lu et al., 2008) and household samples of pesticide residues (Obendorf et al., 2006), seasonal variation in exposure could be misestimated if creatinine corrections were applied. In addition to seasonal variation, we also identified diurnal differences in children's creatinine concentration where morning urinary creatinine levels were usually higher than the evening samples. This finding is consistent with the physiology in which the time from the last void of the day is extended until the next morning void, which allows creatinine to accumulate, and this extended period is during sleep when fluid consumption rarely occurs. Thus, the creatinine level in the morning void is more concentrated. This diurnal pattern of urinary creatinine concentration was recently reported in the US population (Barr et al., 2005).

The most important finding from the present study is the confirmation that urinary creatinine levels increase with age in young children. This finding raises doubt as to the efficacy of applying creatinine concentration to correct urinary output of any chemicals or their metabolites in children's urine. Such a correction may unintentionally introduce bias to the data analysis. For example, given the same exposure level, correcting urinary contaminant concentrations with creatinine will likely artificially inflate a younger child's estimated exposure relative to an older child. To avoid introducing this bias to the data analysis, a recent study (Barr et al., 2005) has suggested that creatinine-adjusted chemical concentrations be compared only within demographic groups; however, our data indicate that for young children, the appropriate reference group is limited to same-aged children.

A statistical remedy to this problem has also been proposed, in which the urinary creatinine concentration is included as a predictor variable in a multiple regression model with the urinary chemical concentration included in the model as either the dependent variable or a separate predictor variable, instead of using the creatinine-adjusted chemical concentration (Barr et al., 2005). Each regression coefficient in the model would represent the independent contribution of the associated predictor variable, after controlling for all other predictor variables in the model, of which, one is urinary creatinine. Unfortunately, this approach complicates the interpretation of the underlying theory on which the model is based. In other words, there is no biological or physiological reason to expect that one's creatinine concentration would be able to predict one's exposure to an environmental chemical; yet, this relationship is being modeled. Furthermore, the underlying theory for creatinine correction is that the blood ratio of creatinine to the target chemical is maintained in urine because both compounds are filtered at the same rate by the kidney. The typical multiplicative approach (dividing the target chemical by the creatinine concentration) maintains this physiological ratio and, as such, is theoretically appropriate. However, in the proposed regression model (Barr et al., 2005), the relationship between creatinine and the target chemical is additive, which is not equivalent to the standard multiplicative correction nor does it reflect the physiological relationship between the two variables.

Attention should be paid to the advantages of using another corrective measure, specific gravity, to account for the differences in urinary dilution. Specific gravity appears to be independent of age and seasonal variation. As was the case for creatinine, the diurnal variation was apparent in the measures of urinary-specific gravity. Again, this pattern accurately reflects physiology. It should be noted that specific gravity may be disproportionately increased when heavy molecules such as glucose and protein are present in the urine; accordingly, neonates and study participants with certain conditions (e.g. diabetes, extreme diet restrictions, dehydration) that alter urinary constituents should be excluded when applying urinary-specific gravity as a corrective factor (Chadha et al., 2001).

Another advantage of measuring specific gravity over creatinine is the simplicity of the analytical method. Creatinine is commonly determined using several different colormetric assays (Fossati et al., 1983; Distasio et al., 1988; Larpent and Verger, 1990; Mei and Wu, 2002; Olsson et al., 2004; Dai, 2007; Keppler et al., 2007), whereas specific gravity can be measured using a handheld refractometer that is easy and inexpensive to use and generates no chemical waste. The primary drawback of measuring specific gravity is that the measurement should be taken when the urine sample is still fresh, not after the sample has been frozen. The freeze–thaw process may modify the content of the dissolvable solids in the urine and can affect the specific gravity measurements.

Conclusion

We have documented systematic variation in urinary creatinine concentrations among young children, discussed

the implication of applying this measure to account for urine concentration in exposure studies, and suggested specific gravity as an alternative to creatinine for correction of urinary output, especially in children. The application of creatinine correction is particularly problematic in studies of young children because it introduces additional bias to the data analysis, including an erroneous increase in younger children's exposure levels as well as seasonal differences in exposure. Specific gravity, on the other hand, is less influenced by person or temporal variables and offers practical advantages over the measurement of creatinine.

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