Published in final edited form as:
*Environ Res*. 2014 July ; 132: 226–232. doi:10.1016/j.envres.2014.04.013.

# Impact of urine concentration adjustment method on associations between urine metals and estimated glomerular filtration rates (eGFR) in adolescents☆

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## Abstract

Positive associations between urine toxicant levels and measures of glomerular filtration rate (GFR) have been reported recently in a range of populations. The explanation for these associations, in a direction opposite that of traditional nephrotoxicity, is uncertain. Variation in associations by urine concentration adjustment approach has also been observed. Associations of urine cadmium, thallium and uranium in models of serum creatinine- and cystatin-C-based estimated GFR (eGFR) were examined using multiple linear regression in a cross-sectional study

☆*Source of funding*: This study was supported by Grant no. R01 ES015597 from the U.S. National Institute of Environmental Health Sciences. The funding sources had no involvement in study design; data collection, analysis and interpretation; manuscript writing; or decisions to submit the work for publication.



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of adolescents residing near a lead smelter complex. Urine concentration adjustment approaches compared included urine creatinine, urine osmolality and no adjustment. Median age, blood lead and urine cadmium, thallium and uranium were 13.9 years, 4.0 μg/dL, 0.22, 0.27 and 0.04 g/g creatinine, respectively, in 512 adolescents. Urine cadmium and thallium were positively associated with serum creatinine-based eGFR only when urine creatinine was used to adjust for urine concentration ($\beta$ coefficient=3.1 mL/min/1.73 $m^2$; 95% confidence interval=1.4, 4.8 per each doubling of urine cadmium). Weaker positive associations, also only with urine creatinine adjustment, were observed between these metals and serum cystatin-C-based eGFR and between urine uranium and serum creatinine-based eGFR. Additional research using non-creatinine-based methods of adjustment for urine concentration is necessary.

**Keywords**

Cadmium; Creatinine; Osmolality; Uranium; Urine concentration

## 1. Introduction

Positive associations between urine nephrotoxicant levels and measures of estimated glomerular filtration rate (eGFR) have been reported recently in a range of populations (de Burbure et al., 2006; Ferraro et al., 2010; Shelley et al., 2012; Weaver et al., 2011b; You et al., 2011). The direction of these associations is contrary to what is expected in nephrotoxicity, despite the fact that some of these toxicants, such as lead and cadmium, have well established nephrotoxic effects. The underlying cause of these associations is currently unknown although variation by biomarker (serum creatinine- versus cystatin C-based eGFR) (Weaver et al., 2011b) and/or estimating equation (You et al., 2011) have been observed. Unexpected associations by urine concentration adjustment method have also been reported. For instance, higher urine uranium was associated with lower measured creatinine clearance (thus, consistent with nephrotoxicity) if urine uranium was adjusted for urine concentration using urine creatinine but not using total uranium excreted over the four-hour collection period (Shelley et al., 2014). Differences in results by urine concentration adjustment method have also been reported in cadmium research using albuminuria and urinary alpha-1-microglobulin as kidney biomarkers (Akerstrom et al., 2013b).

Several mechanisms may explain these paradoxical associations including an impact of kidney processing on urinary toxicant and/or kidney biomarker levels; and statistical associations based on use of urine creatinine to adjust for urine concentration in models of serum creatinine-based outcomes. Research using multiple kidney outcome biomarkers and urine concentration adjustment approaches is needed to unravel these complex results.

The “*Cuida tu Corazon*” (C2C) study provides an ideal population in which to continue such research. Participants include adolescents who are long term residents near the Met-Mex Penoles smelter complex in Torreón, Mexico, which is the fourth largest lead-zinc-silver smelter complex in the world. Residents in Torreón have been exposed to pollution from the smelter complex, including lead and cadmium, for decades (Benin et al., 1999; Garcia Vargas et al., 2001; Soto-Jimenez and Flegal, 2011). Environmental remediation programs have reduced exposure levels over the past two decades as evidenced by results of blood

lead monitoring programs for exposed residents since the late 1990s (Recio-Vega et al., 2012). In addition to measurement of multiple metals in urine samples from the study population to evaluate this mixed exposure setting, we measured serum creatinine and cystatin C as kidney filtration biomarkers and urine osmolality and creatinine as markers of urine concentration.

Our objective was to compare and contrast associations of urine cadmium, thallium and uranium, adjusted for urine concentration with a range of methods, with serum creatinine- and cystatin-C-based GFR in this unique population of adolescents.

## 2. Materials and methods

### 2.1. Study overview and design

The study is a cross-sectional analysis of data from the "*Cuida tu Corazon*" (C2C) study, conducted between October 2009 and June 2010, which was designed to assess levels of lead, cadmium, and arsenic in exposed adolescents and examine their associations with cardiovascular and kidney outcomes. The study was approved by the Institutional Review Boards of the Juarez University of Durango State, the New York State Department of Health and the Johns Hopkins Bloomberg School of Public Health. Participation in the study was voluntary. All subjects and their parents or legal guardians provided written informed consent.

### 2.2. Study population

The C2C study enrolled adolescents who were long-term residents of Torreón, Mexico. Study participants were selected from among the 6254 individuals who had a blood lead measurement prior to 2004 as part of the census-based blood lead surveillance program in Torreón (when the program was run by the Ministry of Health with involvement of one co-author [GGGV]) and who would be 11–16 years of age at the time of the C2C study. This group was stratified into 5 categories based on the first blood lead determination available for each participant in the surveillance program (<10.0, 10.0–14.9, 15.0–19.9, 20.0–44.9, and >45.0 μg/dL). Simple random sampling was performed within each stratum until a target sample size of 512 participants who had been living in the same location since the initial blood lead determination was identified. The participation rate among eligible subjects was 81.9%

### 2.3. Data collection

Data were collected via two home visits and one C2C study clinic visit. Data on sociodemographic factors were collected from the main caregiver via questionnaires by trained study personnel. During the clinic visit, additional data on tobacco use, secondhand smoke exposure, and medication history were elicited privately from each participant. Anthropometric and blood pressure measurements were obtained. Overnight fasting blood samples were collected in heparinized tubes for blood lead and in red top tubes for serum creatinine and cystatin C measurements. Spot urine samples were collected in plastic containers that had been washed with 10% (v/v) $HNO_3$ overnight and rinsed with deionized water. Blood and urine samples were immediately refrigerated and transported in portable

freezers to the Laboratory of Toxicology of Juárez University of Durango State, where samples were aliquoted into 2 mL cryovials (Corning Life Sciences, USA) and frozen at −80 °C.

### 2.4. Metals exposure assessment

Urine metal concentrations were measured at the Trace Elements section of the Laboratory of Inorganic and Nuclear Chemistry at the New York State Department of Health's Wadsworth Center (Albany, New York, USA), which is the principal New York State reference laboratory for trace metals measurement. The analyses were carried out using an ELAN DRC II inductively coupled plasma-mass spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, Connecticut, USA) equipped with Dynamic Reaction Cell (DRC-ICP-MS) technology (Minnich et al., 2008). This multi-element method has been validated against the National Institute of Standards and Technology (NIST) Standard Reference Materials 2670a Toxic Elements in Urine (freeze-dried) and 2668 Toxic Elements in Frozen Human Urine, as well as secondary reference materials from a number of External Quality Assessment Schemes in which the lab participates successfully, including those operated by the Institut National de Santé Publique du Québec, Centre de Toxicologie du Québec, Canada, the Friedrich-Alexander University, Erlangen, Germany, and the University of Surrey, Guildford, UK Trace Elements scheme. As previously described (Minnich et al., 2008; Shelley et al., 2012, 2014; Weaver et al., 2011a), 500 µL of urine was diluted 1 + 19 with 2% (v/v) $HNO_3$ produced in-house using a DuoPUR sub-boiling acid still (Milestone, Shelton, CT, USA), 0.005% Triton X-100 as a surfactant (Sigma-Aldrich, St Louis, MO), 1 mg/L gold and 10 µg/L gallium, rhodium, yttrium, and iridium (High-Purity Standards, Charleston, SC, USA) as internal standards. Multi-element calibration standards were prepared by serial dilution of NIST-traceable stock solution (High Purity Standards, Charleston, SC) using a six point calibration curve for each element. Base human urine pools were used to matrix-match the calibration standards. Samples were prepared under conditions (Clean Room and Class IIB Biological Safety Cabinet) certified as Class 100 or better to minimize the potential for contamination.

Quality control during the course of the study included analysis of urine-based internal quality control (IQC) materials before, during and after every analytical run. The mean coefficients of variation (CV) of the IQC samples from 15 days over the 1-month period in which the Torreón samples were assayed were 7% at 0.39 µg/L ($n$ = 72), 4% at 1.4 µg/L ($n$ = 71), and 3% at 14 µg/L ($n$ = 63) for cadmium. Corresponding values were 3% at 0.53 µg/L ($n$ = 72), 2% at 1.6 µg/L ($n$ = 71), and 2% at 18 µg/L ($n$ = 63) for thallium and 7% at 0.04 µg/L ($n$ = 72), 4% at 0.13 µg/L ($n$ = 71), and 2% at 1.5 µg/L ($n$ = 63) for uranium. The LODs for cadmium, thallium, and uranium were 0.02, 0.02, and 0.001 µg/L, respectively. The corresponding numbers of participants with urine element levels < LOD were 4 (0.8%), 2 (0.4%), and 1(0.2%). Median CVs from duplicate analyses (e.g., inter-assay CV) were 4.2% ($n$ = 75); 2.3% ($n$ = 75); and 5.1% ($n$ = 76) for cadmium, thallium and uranium, respectively. Details of cadmium correction for potential polyatomic interference from molybdenum were as previously published (Weaver et al., 2011a).

Concentrations of lead in whole blood were measured in duplicate at the Laboratory of Toxicology of Juárez University of Durango State using a graphite furnace atomic absorption spectrometer equipped with Zeeman background correction (Analyst 800, Perkin Elmer Norwalk, CT) (Miller et al., 1987). The limit of detection was 0.7 μg/dL. Mean CV of all analyzed specimens was 3.9%, samples with a CV > 5% were reanalyzed ($n$ = 11). For external quality control, the laboratory successfully participates in the blood lead Inter-Laboratory Program of Quality Control from the Faculty of Medicine, University of Zaragoza, Zaragoza, Spain and in the Wisconsin State Laboratory of Hygiene's Proficiency Testing Program for blood lead.

### 2.5. Urine concentration measurements

Urine creatinine concentrations were measured via a Dimension clinical chemistry system using a Flex reagent cartridge in an enzymatic assay (Siemens Dimension Vista 1500; Siemens Medical Solutions USA, Inc., Malvern, PA, United States). Urine osmolality concentrations were measured via an osmometer utilizing the freezing point depression method (Model 3250; Advanced Instruments, Inc., Norwood, MA, US; www.aicompanies.com). For quality control (QC) purposes, urine creatinine and osmolality results were ordered by concentration and five percent was selected sequentially for duplication (28 and 26 samples repeated, respectively). Median CVs were 2.3 and 0.2%, respectively.

### 2.6. Kidney outcome assessment

Serum creatinine concentrations were measured via a Dimension clinical chemistry system using a Flex reagent cartridge in an isotope dilution mass spectrometry (IDMS) traceable enzymatic assay (model RxL; Dade Behring, Glasgow, Delaware, USA) based on NIST standards. Serum cystatin C was measured using an automated Dade Behring nephelometry assay on a Dimension Vista Lab System (Siemens Healthcare Diagnostics, Deerfield, IL, USA). For QC purposes, the original serum creatinine and cystatin C results were ordered by concentration and five percent was selected sequentially for duplication (26 and 27 samples repeated, respectively). Median CVs were 2.8 and 1.8%, respectively.

Estimated GFRs were calculated with the “bedside” Chronic Kidney Disease in Children Prospective Cohort Study (CKiD) equation (Schwartz et al., 2009), based on serum creatinine, and the Filler equation (Filler and Lepage, 2003), based on serum cystatin C, as follows:

- 0.413*[height in cm/serum creatinine in mg/dL]
- $91.62*[(1/\text{serum cystatin C in mg/L})^{1.123}]$

### 2.7. Statistical analysis

The goal of the statistical analyses was to compare and contrast a range of urine concentration adjustment methods for three urine metals, cadmium, thallium and uranium, in models of GFR estimated with two different biomarkers, serum creatinine and cystatin C. All statistical analyses were performed using Stata 12 (Stata Corp, College Station, TX).

Initially, variable distributions were examined. Blood lead and urine metal concentrations were skewed and were log transformed to minimize influential datapoints. We calculated Spearman rank correlation coefficients between exposure variables, urine concentration adjustment factors and the two eGFR measures. Multiple linear regression was performed in models with serum creatinine- or cystatin-C-based eGFR as dependent variables. Metal concentrations were analyzed categorically as quartiles and as continuous log base 2 transformed variables. Three approaches were used to adjust for urine concentration: no adjustment, urine creatinine, and urine osmolality. Categorical metal variables were created based on metal levels divided by the urine adjustment factor (e.g., μg cadmium/g urine creatinine). *P*-values for linear trend were obtained by entering the medians corresponding to each quartile of the urine concentration adjusted metal distribution as a continuous variable in the regression models (Agresti, 2002). In models in which the metals were analyzed as continuous variables, urine creatinine or osmolality and log base 2 transformed urine metals were entered as separate covariates (Barr et al., 2005). Models were progressively adjusted from crude to full which included age, sex, body mass index (BMI; weight in kilograms divided by the square of height in meters), maternal education (none/primary, secondary, > secondary), monthly household income (< 3000 pesos, ≥ 3000 pesos, unknown/no response), participant smoking status (never, former [not in past month], current [at least once in past month]), systolic blood pressure and natural log transformed blood lead.

Sensitivity analyses included: (1) the log base 2 transformation of each urine metal concentration (μg/L) divided by urine creatinine (mg/dL) or urine osmolality (μOsm/kg) was entered as a single variable in continuous models (e.g., ln urine cadmium μg/g creatinine/ln[2]), and (2) metal quartiles associations were examined in models of serum creatinine and cystatin C (without using an estimating equation for GFR).

Models were evaluated for linear regression assumptions and the presence of influential datapoints using augmented component-plus-residual plots, added-variable plots, and leverage versus residual plots (Chen et al., 2003; Weisberg, 1985) and repeated with outlying and leverage datapoints removed when applicable. The same datapoints were removed in the various continuous models of each exposure so that populations were comparable. Normality assumptions were also assessed with Q–Q and Kernel density plots. Heteroskedasticity was assessed with the hettest command and, if significant, model standard errors were recalculated with the STATA hc3 command. Models were also assessed for co-linearity through examination of variance inflation factors, all of which were below 3.

## 3. Results

Mean age of the 512 adolescent participants was 14.0 (range 11.9–16.0) years and 262 (51%) were male (Table 1). Sixty-one percent were from households with monthly incomes of less than 3000 pesos and 60% were never-smokers. Median BMI was 20.5 kg/m$^2$. Median blood lead and urine cadmium, thallium, and uranium levels were 4.0 μg/dL and 0.22, 0.27 and 0.04 μg/g creatinine, respectively. Mean serum creatinine- and cystatin-C-based eGFR were 112.5 and 126.1 mL/min/1.73 m$^2$, respectively.

Urine creatinine was positively correlated with urine osmolality and with all three metals before adjustment for urine concentration and after adjustment with urine osmolality (Table 2). Urine creatinine was negatively correlated with all three metals in which it was used to adjust for urine concentration. Urine osmolality was also positively correlated with all three urine metals before adjustment and negatively correlated after adjustment with urine creatinine but not correlated after adjustment with urine osmolality. Urine creatinine and osmolality were both negatively correlated with serum creatinine-based eGFR but neither was associated with serum cystatin-C-based eGFR The eGFR measures were not highly correlated ($r_s$=0.3). Concentrations of each metal, adjusted for urine creatinine and osmolality, were highly correlated ($r_s$=0.76, 0.62 and 0.81 for cadmium, thallium and uranium, respectively). Correlations between different metals varied widely from −0.13 for cadmium (μg/L) and thallium (μg/g creatinine) to 0.56 for thallium and uranium (both in μg/L).

### 3.1. Associations of urine metals with kidney outcomes

In fully adjusted models in which metals were divided by urine creatinine and entered as quartiles (Table 3, middle column), cadmium and thallium were positively associated with both serum creatinine- and cystatin-C-based eGFR measures and the positive association of urine uranium approached significance in the model of serum creatinine-based eGFR In contrast, urine uranium unadjusted for urine concentration was negatively associated with serum creatinine-based eGFR. A negative association, although non-significant (*p*-trend=0.07), was also observed for uranium adjusted with urine osmolality (Table 3, last column). No other associations were observed with metals adjusted with urine osmolality.

In models in which urine metals and urine creatinine were entered as separate continuous variables (Table 4, middle column), cadmium and thallium were positively associated with serum creatinine-based eGFR. Cadmium and thallium were also positively associated with cystatin-C-based eGFR, however, associations were weaker. In contrast, when metals were either unadjusted for urine concentration or adjusted with urine osmolality, no significant associations were observed (the $\beta$ coefficient for the association between serum creatinine-based eGFR and thallium adjusted with urine osmolality is influenced upward by the highest and lowest thallium values). As in Table 3, uranium associations were different than the other two metals. Uranium was not associated with serum cystatin-C-based eGFR but, when adjusted for urine creatinine, was borderline positively associated with serum creatinine-based eGFR and, when unadjusted or adjusted with urine osmolality, was borderline negatively associated with serum creatinine-based eGFR. For these latter negative associations, 95% confidence intervals excluded zero with removal of approximately 10 influential high and low uranium values (data not shown).

### 3.2. Sensitivity analyses

In models in which urine metals were divided by either urine creatinine or osmolality and then log base 2 transformed (Table 5), results were similar to Table 4 with the exception of the higher $\beta$ coefficients observed between thallium adjusted for urine creatinine and the two eGFR measures. A similar increase in this association was also observed when natural log transformed urine creatinine was entered as a separate co-variate in the thallium models in

Table 4. Associations of cadmium, thallium and uranium, entered as quartiles, in models of serum creatinine and cystatin C (without using an estimating equation for GFR) were consistent with models in which eGFR was used (Table 6).

## 4. Discussion

We examined associations of urine cadmium, thallium and uranium levels with eGFR measures based on two different biomarkers, serum creatinine and cystatin C. We employed a range of methods to adjust for urine concentration in separate models: each urine metal concentration divided by urine creatinine or osmolality; each metal along with urine creatinine or osmolality entered as separate co-variates; and no adjustment. We observed two key findings. First, associations differed greatly by method of adjustment for urine concentration. Urine creatinine adjusted cadmium and thallium had significant or borderline significant positive associations with eGFR as did uranium with serum creatinine-based eGFR measures. In contrast, the only association observed with urine osmolality adjustment was higher uranium with lower serum creatinine-based eGFR, of borderline significance. The second key finding was the positive direction of the associations with metals adjusted for urine creatinine, which is opposite that expected with nephrotoxicity.

The metals in this analysis were selected because they are released from the smelter complex and/or present in local drinking water sources. Furthermore, these metals are potential or known nephrotoxicants (Shelley et al., 2012, 2014; Weaver et al., 2011a, 2011b). However, no consistent evidence of nephrotoxicity was observed in this analysis. Instead, metals were positively associated with eGFR measures, a finding observed in other recent reports, all using urine creatinine adjustment for urine concentration differences (de Burbure et al., 2006; Ferraro et al., 2010; Shelley et al., 2012; Weaver et al., 2011b; You et al., 2011). Traditionally, urine creatinine or timed urine collections have been used to adjust for variation in urine biomarker concentrations due to underlying differences in urine concentration/dilution. Twenty-four hour urine samples may be the “gold standard” but are difficult to comply with and have the potential for external contamination. Creatinine varies by sex, age and diet due to its metabolism from creatine in muscle. Other urine concentration adjustment options, such as specific gravity, have also been explored but the optimal adjustment approach remains uncertain (Barr et al., 2005; Boeniger et al., 1993; Suwazono et al., 2005).

Research comparing different approaches to urine concentration adjustment has yielded both consistent and inconsistent results. A study in Swedish women reported no major differences between urine cadmium associations, adjusted with urine creatinine or specific gravity, and serum creatinine- and cystatin-C-based glomerular filtration measures (Akesson et al., 2005). Kurttio et al. reported no associations between creatinine unadjusted urine uranium (μg/L) and serum cystatin C or measured creatinine clearance, noting that results were similar with creatinine-corrected urinary uranium (μg/g creatinine) and uranium exposure measures that did not require adjustment for urine concentration (e.g., uranium in hair and toenails) (Kurttio et al., 2006). In another study in adolescents, the associations between urine cadmium and lead with early biomarkers of kidney effects in urine (albumin, β-2-microglobulin and retinol-binding protein), did not differ by urine creatinine and specific

gravity adjustment (Chaumont et al., 2012). However, the authors concluded that neither urine creatinine nor specific gravity adequately adjusted for urine concentration based on persistent correlations with adjusted metals. Urine concentration adjustment approaches have been compared in exposure biomonitoring research as well. For example, associations between urine cotinine, adjusted for creatinine or specific gravity, and blood cotinine were reported to be comparable in 431 participants (Muscat et al., 2011).

Differences in associations by urine concentration adjustment method have also been observed. Shelley et. al. reported an association between urine uranium, adjusted with urine creatinine, and measured creatinine clearance but this was not observed when urine concentration adjustment was addressed by using total uranium excreted in a timed urine sample as the exposure metric (Shelley et al., 2014). Correlations between kidney early biological effect markers in urine, including retinol binding protein and N-acetyl-beta-D-glucosaminidase, and urine cadmium adjusted with either urine creatinine or specific gravity were lower as compared to correlations determined without urine concentration adjustment (Moriguchi et al., 2003). In another population, associations between urine kidney early biologic effect markers and urine cadmium were stronger using urine cadmium excretion rates and adjustment with specific gravity rather than urine creatinine (Akerstrom et al., 2013b). However, in biomonitoring research, a stronger correlation between cadmium levels in kidney and urine was observed with adjustment for creatinine rather than specific gravity or overnight urinary excretion rate, in 109 living kidney donors (Akerstrom et al., 2013a).

Thus, although some inconsistencies by urine concentration adjustment approach have been reported in the published literature, differences as striking as that observed herein are not common. However, osmolality is rarely used as a urine concentration adjustor despite the fact that the impact of large molecular weight molecules (e.g. glucose or albumin) is less on osmolality than specific gravity. In particular, osmolality may be especially useful in children and adolescents since creatinine varies greatly by age and orthostatic proteinuria in adolescents would have a greater impact on specific gravity than on osmolality.

The mechanism(s) for the positive associations between metals and eGFR, observed only with urine creatinine adjustment in this study, remains uncertain. If positive associations were due to reverse causality from reduced excretion in chronic kidney disease or to normal glomerular filtration in which urine metal levels increase as eGFR does, the associations should be similar after urine concentration adjustment using either urine creatinine or osmolality. The differences by urine concentration adjustor also make hyperfiltration (e.g., the positive correlation between measured GFR and blood lead observed in an experimental animal model of lead exposure (Khalil-Manesh et al., 1992)) less likely. However, kidney processing of creatinine, as discussed below, or of urine, thus impacting osmolality, may be different and/or affected by metal levels so we cannot completely exclude these mechanisms.

Metals may affect kidney processing of creatinine. In addition to being filtered by the glomerulus, data suggest that creatinine is secreted in the proximal tubule of the kidney via both organic cation and anion transporters (Ciarimboli et al., 2012; Eisner et al., 2010; Urakami et al., 2004). in vitro data indicate that cadmium increases transport of p-

aminohippurate (an organic anion substrate) at lower levels but inhibits it at higher levels (Van Kerkhove et al., 2010). Recent data also indicate that cadmium crosses the basolateral membrane of the renal proximal tubular cells (Soodvilai et al., 2011) via the same organic cation transporters used for creatinine. Uranium transport mechanisms are not well defined although an impact on substrates of both organic anion and cation transporters was observed in an experimental animal model of uranyl nitrate-induced acute renal failure (Tanigawara et al., 1990).

Another possible explanation for the positive associations observed with urine creatinine adjustment is that, in nephrotoxicant research, outcome variables often contain the same or correlated creatinine values. For example, in the data herein, the Spearman correlation coefficient between serum creatinine, used in the denominator of the serum creatinine-based eGFR, and urine creatinine, also in the denominator of the metals in Table 5, was 0.35 ($p < 0.001$). Thus, the potential for a statistical rather than a biological positive association exists. However, the positive associations with serum cystatin-C-based eGFR, although weaker, are not supportive of this hypothesis. The differences in uranium associations compared to cadmium and thallium also suggest unique metal-specific mechanisms, such as differing impacts on transporters or metal–protein binding affecting excretion (Chaumont et al., 2012). Thus, more than one process may be involved in these complex findings.

Strengths of this study include the census-based source population comprised of adolescents, an understudied age group in environmental epidemiology, standardized study questionnaires and protocols, high quality laboratory methods and large sample size. In addition, the study obtained data on numerous co-variates, including multiple metals so that environmental exposures to mixtures, which is common, could be investigated. Limitations include the cross-sectional study design which does not allow the temporality of the associations to be addressed. Also, GFR was estimated rather than measured and optimal estimating equations in children and adolescents with normal range kidney function remain a research topic.

## 5. Conclusions

In conclusion, urine cadmium and thallium were positively associated with serum creatinine-based eGFR only when urine creatinine was used to adjust for urine concentration. Weaker positive associations, also only with urine creatinine adjustment, were observed between urine cadmium and thallium with serum cystatin-C-based eGFR and between urine uranium and serum creatinine-based eGFR. The positive direction was opposite that expected with nephrotoxicity. Additional research using multiple kidney outcome biomarkers and non-creatinine-based methods of adjustment for urine concentration, such as osmolality or timed urine samples, as well as non-urine based exposure measures (e.g., blood levels) is needed.

## Acknowledgments

We thank the Ministry of Health of Coahuila State, Mexico, for the facilities used in this study.

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## Abbreviations

| | |
|---|---|
| **BMI** | body mass index |
| **C2C** | Cuida tu Corazón (Take Care of your Heart) |
| **CV** | coefficient of variation |
| **DRC-ICP-MS** | dynamic reaction cell inductively coupled plasma-mass spectrometer |
| **eGFR** | estimated glomerular filtration rate |
| **$HNO_3$** | nitric acid |
| **IQC** | internal quality control |
| **MDL** | method detection limit |
| **NIST** | National Institute of Standards and Technology |
| **SD** | standard deviation |

## Table 1

Selected participant characteristics (*N*=512).

| Variables | *N* (%) | |
|---|---|---|
| Male | 262 (51.2) | |
| Maternal education | | |
| None or Primary | 186 (36.3) | |
| Secondary | 206 (40.2) | |
| >Secondary | 120 (23.4) | |
| Monthly household income | | |
| <3000 pesos | 311 (60.7) | |
| ≥3000 pesos | 144 (28.1) | |
| Unknown/no response | 57 (11.1) | |
| Smoking status | | |
| Never | 308 (60.2) | |
| Former | 149 (29.1) | |
| Current | 55 (10.7) | |
| | **Median** | **Mean (SD)** |
| Age, years | 13.9 | 14.0 (1.1) |
| Body mass index, kg/m$^2$ | 20.5 | 21.7 (4.9) |
| Height, cm | 158.5 | 158.8 (8.3) |
| Systolic blood pressure, mm Hg | 104 | 105.3 (9.1) |
| Serum creatinine eGFR, mL/min/1.73 m$^2$ | 111.4 | 112.5 (17.8) |
| Serum cystatin C eGFR, mL/min/1.73 m$^2$ | 124.8 | 126.1 (19.7) |
| Blood lead, μg/dL | 4.0 | 4.6 (2.8) |
| Urine cadmium, μg/L | 0.24 | 0.34 (0.37) |
| Urine cadmium, μg/g creatinine | 0.22 | 0.29 (0.38) |
| Urine cadmium, μg/μOsm/kg | 0.33 | 0.47 (0.62) |
| Urine thallium, μg/L | 0.33 | 0.35 (0.20) |
| Urine thallium, μg/g creatinine | 0.27 | 0.31 (0.18) |
| Urine thallium, μg/μOsm/kg | 0.42 | 0.46 (0.21) |
| Urine uranium, μg/L | 0.04 | 0.07 (0.14) |
| Urine uranium, μg/g creatinine | 0.04 | 0.07 (0.29) |
| Urine uranium, μg/μOsm/kg | 0.06 | 0.12 (0.44) |
| Urine creatinine, mg/dL | 118.5 | 131.9 (79.3) |
| Urine osmolality, μOsm/kg | 0.82 | 0.76 (0.26) |

**Table 2**

Spearman rank correlation coefficients for urine concentration adjustment variables, eGFR measures and metal levels, in 512 adolescents.

| | Urine creatinine (mg/dL) | Osmo-lality (µOsm/kg urine) | Serum creatinine-based eGFR | Serum cystatin-C-based eGFR | Cadmium (µg/L) | Cadmium (µg/g) | Cadmium (µg/µOsm/kg) | Thallium (µg/L) | Thallium (µg/g) | Thallium (µg/µOsm/kg) | Uranium (µg/L) | Uranium (µg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Osmolality, µOsm/kg urine | 0.79[c] | | | | | | | | | | | |
| Creatinine-based eGFR, mL/min/1.73 $m^2$ | −0.32[c] | −0.17[a] | | | | | | | | | | |
| Cystatin-C-based eGFR, mL/min/1.73 $m^2$ | −0.07 | −0.01 | 0.30[c] | | | | | | | | | |
| Cadmium, µg/L | 0.61[c] | 0.52[c] | −0.12 | −0.07 | | | | | | | | |
| Cadmium, µg/g creatinine | −0.33[c] | −0.24[c] | 0.19[c] | 0.05 | 0.48[c] | | | | | | | |
| Cadmium, µg/µOsm/kg urine | 0.17[a] | −0.03 | −0.07 | −0.02 | 0.76[c] | 0.76[c] | | | | | | |
| Thallium, µg/L | 0.61[c] | 0.62[c] | −0.08 | −0.13 | 0.53[c] | −0.04 | 0.15 | | | | | |
| Thallium, µg/g creatinine | −0.47[c] | −0.22[c] | 0.32[c] | −0.05 | −0.13 | 0.32[c] | −0.09 | 0.33[c] | | | | |
| Thallium, µg/µOsm/kg urine | 0.18[b] | 0.07 | 0.02 | −0.14 | 0.29[c] | 0.16[a] | 0.27[c] | 0.77[c] | 0.62[c] | | | |
| Uranium, µg/L | 0.63[c] | 0.44[c] | −0.13 | −0.14 | 0.49[c] | −0.07 | 0.25[c] | 0.56[c] | −0.12 | 0.39[c] | | |
| Uranium, µg/g creatinine | −0.18[b] | −0.22[c] | 0.16[a] | −0.06 | −0.03 | 0.24[c] | 0.15 | 0.06 | 0.26[c] | 0.27[c] | 0.59[c] | |
| Uranium, µg/µOsm/kg urine | 0.27[c] | −0.02 | −0.08 | −0.10 | 0.23[c] | 0.07 | 0.35[c] | 0.23[c] | −0.11 | 0.35[c] | 0.83[c] | 0.81[c] |

[a] *p*-Value<0.05.

[b] *p*-Value<0.01.

[c] *p*-Value<0.001 with Bonferroni correction.

## Table 3

Mean difference in eGFR measures, in mL/min/1.73 $m^2$, by quartiles of urine metal concentrations, using three approaches to urine concentration adjustment.

| | Urine concentration adjustment method | | |
|---|---|---|---|
| | **μg/L β (95% CI)** | **μg/g Creatinine β (95% CI)** | **μg/μOsm/kg β (95% CI)** |
| **Cadmium** | **CKiD bedside serum creatinine-based eGFR** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | −0.3 (−4.2, 3.5) | 3.5 (−0.4, 7.3) | −0.7 (−4.6, 3.3) |
| **Q3** | −0.8 (−4.8, 3.1) | 7.8 (3.9, 11.8) | −1.1 (−5.1, 2.9) |
| **Q4** | −1.1 (−5.2, 3.1) | 7.2 (3.0, 11.4) | −1.2 (−5.3, 2.9) |
| *p*-Trend[a] | 0.62 | 0.001 | 0.62 |
| **Cadmium** | **Filler serum cystatin-C-based eGFR** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | 2.0 (−2.0, 6.0) | 1.8 (−2.3, 5.8) | 2.6 (−1.5, 6.7) |
| **Q3** | 0.5 (−3.6, 4.6) | 5.9 (1.8, 10.0) | 1.9 (−2.2, 6.1) |
| **Q4** | 0.7 (−3.6, 5.1) | 4.9 (0.4, 9.3) | −0.4 (−4.7, 3.9) |
| *p*-Trend | 0.99 | 0.03 | 0.47 |
| **Thallium** | **CKiD bedside serum creatinine-based eGFR** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | 2.9 (−1.0, 6.8) | 1.2 (−2.5, 5.0) | 2.3 (−1.6, 6.3) |
| **Q3** | 0.7 (−3.2, 4.7) | 6.5 (2.7, 10.2) | 2.6 (−1.3, 6.5) |
| **Q4** | 0.0 (−4.0, 4.1) | 10.9 (7.0, 14.8) | 1.3 (−2.8, 5.3) |
| *p*-Trend | 0.71 | <0.001 | 0.67 |
| **Thallium** | **Filler serum cystatin-C-based eGFR** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | 0.6 (−3.5, 4.7) | 0.3 (−3.8,4.3) | 1.3 (−2.8, 5.4) |
| **Q3** | −1.8 (−5.9, 2.3) | 2.9 (−1.1, 7.0) | −1.3 (−5.3, 2.8) |
| **Q4** | 1.0 (−3.2, 5.2) | 4.4 (0.2,8.6) | 0.4 (−3.8, 4.6) |
| *p*-Trend | 0.80 | 0.02 | 0.85 |
| **Uranium** | **CKiD bedside serum creatinine-based eGFR** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | 0.5 (−3.4, 4.4) | 1.9 (−2.0, 5.8) | −1.8 (−5.6, 2.1) |
| **Q3** | −0.7 (−4.6, 3.3) | 2.9 (−1.1, 6.9) | −1.4 (−5.3, 2.6) |
| **Q4** | −3.7 (−7.7, 0.3) | 3.8 (−0.3, 7.9) | −3.9 (−7.8, 0.1) |
| *p*-Trend | 0.03 | 0.08 | 0.07 |
| **Uranium** | **Filler serum cystatin-C-based eGFR** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | 0.9 (−3.2, 5.0) | 0.4 (−3.7, 4.5) | −0.4 (−4.4, 3.7) |
| **Q3** | −2.4 (−6.5,1.7) | 1.5 (−2.7, 5.7) | −4.9 (−9.0, −0.8) |
| **Q4** | −1.2 (−5.4, 2.9) | 1.5 (−2.8, 5.8) | −1.8 (−6.0, 2.3) |
| *p*-Trend | 0.39 | 0.46 | 0.36 |

Adjusted for age, sex, maternal education (none/primary, secondary, >secondary), monthly household income (<3000 pesos, ≥3000 pesos, unknown/no response), participant smoking status (never, former [not in past month], current [at least once in past month]), BMI, systolic blood pressure and natural log blood lead.

[a] *p*-Value for trend based on Wald test from regression model in which quartile medians were entered.Quartiles 1, 2 and 3 cut-points are as follows: <0.149, <0.237, and <0.371 for cadmium in μg/L; <0.149, <0.220, and <0.333 for cadmium in μg/g creatinine; <0.233, <0.328, and <0.511 for cadmium in μg/μOsm/kg; <0.209, <0.332, and <0.453 for thallium in μg/L; <0.192, <0.269, <0.378 for thallium in μg/g creatinine; <0.318, <0.417, and <0.550 for thallium in μg/μOsm/kg; <0.025, <0.044, and <0.076 for uranium in μg/L; <0.026, <0.042, and <0.061 for uranium in μg/g creatinine; <0.039, <0.062, and <0.098 for uranium in μg/μOsm/kg.*β* reflects the difference in serum creatinine and cystatin C comparing metal quartiles 2–4 to the lowest quartile.

## Table 4

Mean difference in eGFR measures, in mL/min/1.73 $m^2$, per each doubling of urine metal concentration, by urine concentration adjustment approach.

| | Urine concentration adjustment method | | |
|---|---|---|---|
| | $\beta$ (95% CI) None | $\beta$ (95% CI) Urine creatinine (mg/dL) | $\beta$ (95% CI) Urine osmolality (μOsm/kg) |
| **Cadmium** | **CKiD bedside serum creatinine-based eGFR** | | |
| Model 1 | −1.5 (−2.9, −0.1) | 2.3 (0.6, 3.9) | −0.7 (−2.3, 1.0) |
| Model 2 | −0.1 (−1.4, 1.2) | 3.0 (1.4, 4.5) | 0.4 (−1.1, 1.9) |
| Model 3 | −0.1 (−1.4, 1.2) | 2.8 (1.3, 4.4) | 0.3 (−1.2, 1.8) |
| Model 4 | −0.3 (−1.6, 1.1) | 3.1 (1.4, 4.8) | 0.1 (−1.5, 1.7) |
| | **Filler serum cystatin-C-based eGFR** | | |
| Model 1 | −1.4 (−2.9, 0.1) | −1.0 (−3.0, 0.9) | −1.2 (−3.0, 0.6) |
| Model 2 | −0.4 (−1.7, 1.0) | 0.4 (−1.3, 2.1) | −0.9 (−2.5, 0.7) |
| Model 3 | −0.4 (−1.7, 1.0) | 0.4 (−1.3, 2.1) | −1.0 (−2.6, 0.6) |
| Model 4 | 0.4 (−1.0, 1.8) | 1.8 (0.0, 3.7) | 0.0 (−1.7, 1.7) |
| **Thallium** | **CKiD bedside serum creatinine-based eGFR** | | |
| Model 1 | −1.1 (−2.6, 0.5) | 3.2 (1.5, 5.0) | 1.9 (−0.3, 4.1) |
| Model 2 | −0.1 (−1.5, 1.4) | 3.6 (1.9, 5.3) | 2.0 (−0.1, 4.0) |
| Model 3 | 0.1 (−1.4,1.5) | 3.6 (1.9, 5.3) | 1.9 (−0.2, 4.0) |
| Model 4 | −0.0 (−1.5, 1.5) | 3.6 (1.8, 5.3) | 1.8 (−0.3, 3.9) |
| | **Filler serum cystatin-C-based eGFR** | | |
| Model 1 | −2.6 (−4.2, −0.9) | −2.7 (−4.8, −0.6) | −3.9 (−6.3, −1.4) |
| Model 2 | −0.0 (−1.5, 1.5) | 1.1 (−0.8, 3.0) | −0.8 (−3.0, 1.4) |
| Model 3 | 0.1 (−1.4, 1.7) | 1.3 (−0.6, 3.2) | −0.7 (−2.9, 1.5) |
| Model 4 | 0.5 (−1.1, 2.0) | 1.9 (−0.0, 3.8) | −0.0 (−2.2, 2.2) |
| **Uranium** | **CKiD bedside serum creatinine-based eGFR** | | |
| Model 1 | −1.3 (−2.4, −0.2) | 1.5 (0.2, 2.8) | −0.6 (−1.9, 0.6) |
| Model 2 | −0.9 (−1.9, 0.1) | 1.2 (−0.0, 2.4) | −0.6 (−1.8, 0.5) |
| Model 3 | −0.9 (−2.0, 0.1) | 1.1 (−0.2, 2.4) | −0.8 (−1.9, 0.4) |
| Model 4 | −1.0 (−2.0, 0.1) | 1.1 (−0.2, 2.3) | −0.8 (−2.0, 0.3) |
| | **Filler serum cystatin-C-based eGFR** | | |
| Model 1 | −1.8 (-3.0, −0.6) | −1.9 (−3.4, −0.3) | −1.8 (−3.2, −0.4) |
| Model 2 | −0.3 (−1.4, 0.8) | 0.3 (−1.1, 1.6) | −0.6 (−1.8, 0.6) |
| Model 3 | −0.1 (−1.2, 1.0) | 0.6 (−0.8, 2.0) | −0.4 (−1.7, 0.7) |
| Model 4 | 0.0 (−1.1, 1.1) | 0.9 (−0.5, 2.3) | −0.2 (−1.5, 1.0) |

Model 1: Crude (independent variables include only urine metal [ln (metal)/ln (2)] and urine creatinine or osmolality [added in last two columns] as separate co-variates.

Model 2: Adjusted for Model 1 co-variates, age, and sex.

Model 3: Adjusted for Model 2 co-variates, BMI, maternal education (none/primary, secondary, >secondary), monthly household income (<3000 pesos, ≥3000 pesos, unknown/no response), participant smoking status (never, former [not in past month], current [at least once in past month]), and systolic blood pressure.

Model 4: Adjusted for Model 3 co-variates and natural log blood lead. $\beta$ reflects ml/min/1.73 $m^2$ in eGFR for a doubling in metal concentration.

## Table 5

Associations between eGFR measures and doubling of urine metal/urine creatinine or osmolality.

| | Urine concentration adjustment method | |
|---|---|---|
| | **μg metal/g Urine creatinine β (95% CI)** | **μg metal/μOsm/Kg β (95% CI)** |
| **Cadmium** | **CKiD bedside serum creatinine-based eGFR** | |
| | 3.6 (1.9, 5.3) | −0.4 (−2.0, 1.2) |
| | **Filler serum cystatin-C-based eGFR** | |
| | 2.1 (0.3, 3.8) | −0.0 (−1.7, 1.6) |
| **Thallium** | **CKiD bedside serum creatinine-based eGFR** | |
| | 6.3 (4.4, 8.1) | 1.3 (−1.0, 3.6) |
| | **Filler serum cystatin-C-based eGFR** | |
| | 3.0 (0.9, 5.0) | 0.2 (−2.2, 2.6) |
| **Uranium** | **CKiD bedside serum creatinine-based eGFR** | |
| | 1.2 (−0.2, 2.5) | −1.0 (−2.2, 0.2) |
| | **Filler serum cystatin-C-based eGFR** | |
| | 0.9 (−0.5, 2.3) | −0.3 (−1.5, 0.9) |

Models also adjusted for age, sex, BMI, maternal education (none/primary, secondary, >secondary), monthly household income (<3000 pesos, ≥3000 pesos, unknown/no response), participant smoking status (never, former [not in past month], current [at least once in past month]), systolic blood pressure, systolic blood pressure and natural log blood lead.

$\beta$ reflects ml/min/1.73 $m^2$ in eGFR for a doubling in metal concentration.

## Table 6

Mean difference in serum creatinine and cystatin C by quartiles of urine metal concentrations, using three approaches to urine concentration adjustment.

| | Urine concentration adjustment method | | |
|---|---|---|---|
| | **μg/L β (95% CI)** | **μg/g Creatinine β (95% CI)** | **μg/μOsm/kg β (95% CI)** |
| **Cadmium** | **Serum creatinine** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | −0.00 (−0.02, 0.02) | −0.02 (−0.04, 0.00) | 0.00 (−0.02, 0.03) |
| **Q3** | 0.01 (−0.02,0.03) | −0.06 (−0.08, −0.03) | 0.00 (−0.02, 0.03) |
| **Q4** | −0.00 (−0.02, 0.02) | −0.06 (−0.08, −0.03) | 0.00 (−0.02, 0.03) |
| *p*-Trend[a] | 0.88 | <0.001 | 0.90 |
| **Cadmium** | **Serum cystatin C** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | −0.00 (−0.03, 0.02) | −0.01 (−0.03, 0.02) | −0.01 (−0.03, 0.01) |
| **Q3** | 0.00 (−0.02, 0.02) | −0.03 (−0.05, −0.01) | −0.01 (−0.03, 0.02) |
| **Q4** | −0.00 (−0.03, 0.02) | −0.03 (−0.05, −0.00) | 0.00 (−0.02, 0.02) |
| *p*-Trend[a] | 0.87 | 0.005 | 0.88 |
| **Thallium** | **Serum creatinine** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | −0.01 (−0.03, 0.01) | −0.01 (−0.03, 0.01) | −0.01 (−0.04, 0.01) |
| **Q3** | 0.00 (−0.02, 0.03) | −0.04 (−0.07, −0.02) | −0.01 (−0.04, 0.01) |
| **Q4** | 0.00 (−0.02, 0.03) | −0.08 (−0.10, −0.05) | −0.00 (−0.03, 0.02) |
| *p*-Trend | 0.45 | <0.001 | 0.88 |
| **Thallium** | **Serum cystatin C** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | −0.00 (−0.03, 0.02) | −0.00 (−0.02, 0.02) | −0.01 (−0.03, 0.01) |
| **Q3** | 0.01 (−0.02, 0.03) | −0.01 (−0.04, 0.01) | 0.01 (−0.01, 0.03) |
| **Q4** | −0.01 (−0.03, 0.02) | −0.02 (−0.05, −0.00) | −0.00 (−0.03, 0.02) |
| *p*-Trend | 0.91 | 0.03 | 0.75 |
| **Uranium** | **Serum creatinine** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | −0.01 (−0.03, 0.01) | −0.02 (−0.04, 0.00) | 0.01 (−0.01, 0.03) |
| **Q3** | −0.00 (−0.02, 0.02) | −0.02 (−0.04, 0.01) | 0.00 (−0.02, 0.02) |
| **Q4** | 0.03 (0.00, 0.05) | −0.03 (−0.05, −0.00) | 0.03 (0.01, 0.06) |
| *p*-Trend | 0.02 | 0.04 | 0.02 |
| **Uranium** | **Serum cystatin C** | | |
| **Q1** | 0 (reference) | 0 (reference) | 0 (reference) |
| **Q2** | −0.00 (−0.03, 0.02) | −0.00 (−0.03, 0.02) | 0.00 (−0.02, 0.02) |
| **Q3** | 0.01 (−0.01, 0.03) | −0.01 (−0.03, 0.01) | 0.02 (0.00, 0.05) |
| **Q4** | 0.00 (−0.02, 0.03) | −0.01 (−0.03, 0.01) | 0.01 (−0.02, 0.03) |
| *p*-Trend | 0.48 | 0.28 | 0.27 |

Adjusted for age, sex, maternal education (none/primary, secondary, >secondary), monthly household income (<3000 pesos, ≥3000 pesos, unknown/no response), participant smoking status (never, former [not in past month], current [at least once in past month]), BMI, systolic blood pressure and natural log blood lead.

[a] *p*-Value for trend based on Wald test from regression model in which quartile category values were entered.Quartiles 1, 2 and 3 cut-points are as follows: <0.149, <0.237, and <0.371 for cadmium in μg/L; <0.149, <0.220, and <0.333 for cadmium in μg/g creatinine; <0.233, <0.328, and <0.511 for cadmium in μg/μOsm/kg; <0.209, <0.332, and <0.453 for thallium in μg/L; <0.192, <0.269, <0.378 for thallium in μg/g creatinine; <0.318, <0.417, and <0.550 for thallium in μg/μOsm/kg; <0.025, <0.044, and <0.076 for uranium in μg/L; <0.026, <0.042, and <0.061 for uranium in μg/g creatinine; <0.039, <0.062, and <0.098 for uranium in μg/μOsm/kg.$\beta$ reflects the difference in serum creatinine and cystatin C comparing metal quartiles 2–4 to the lowest quartile.