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Association of Arsenic with Kidney Function in Adolescents and Young Adults: Results from the National Health and Nutrition Examination Survey 2009–2012

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Abstract

Background—Long-term exposure to arsenic is a major public health concern. Emerging evidence suggests adverse health effects even at low levels of exposure. This study examined the association of arsenic exposure with estimated glomerular filtration rate (eGFR) and compared methods of adjustment for urinary dilution in a representative sample of U.S. adolescents and young adults.

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Methods—We performed a cross-sectional study of 1,253 participants ages 12–30 years in the 2009–2012 National Health and Nutrition Examination Survey (NHANES) with available urinary arsenic and eGFR measures. Multivariable linear regression was used to model the association of urinary total arsenic and dimethylarsinate (DMA) with eGFR.

Results—The median urinary total arsenic and DMA concentrations were 6.3 μg/L (IQR 3.3 – 12.7 μg/L) and 3.3 μg/L (IQR 1.7 – 5.7 μg/L), respectively. Median eGFR was 109 mL/min/1.73 m^2. Adjusting arsenic for urine concentration with urinary creatinine, eGFR was 4.0 mL/min/1.73 m^2 higher (95% confidence interval [CI] 1.0 to 7.1 mL/min/1.73 m^2) and 4.3 mL/min/1.73 m^2 higher (95% CI 0.5 to 8.0 mL/min/1.73 m^2) per log-unit increase in total arsenic and DMA, respectively. When using urine osmolality to adjust for urine concentration, a log-unit increase in total arsenic and DMA was associated with a 0.4 mL/min/1.73 m^2 (95% CI –1.8 to 1.1 mL/min/1.73 m^2) and 0.01 (95% CI –1.9 to 1.9 mL/min/1.73 m^2) lower eGFR, respectively.

Conclusions—Discordant associations were observed between arsenic and eGFR levels depending on whether urinary creatinine or osmolality was used to adjust for urine concentration. Further study should be dedicated to validating the best approach to account for urinary dilution in research in toxicants, and this may have implications for all studies which examine urinary biomarkers.

1. INTRODUCTION

Chronic exposure to inorganic arsenic has become a major public health concern throughout the world. Millions of people worldwide are exposed to inorganic arsenic, primarily through contamination of drinking water by natural arsenic deposits (Smedley and Kinniburgh, 2002). The carcinogenic and toxic effects of long-term consumption of water containing high levels of arsenic have been well-described (Chowdhury et al., 2000; Naujokas et al., 2013). The United States (U.S.) Environmental Protection Agency's standard for arsenic concentration in public drinking water supply is 10 μg/L, and approximately 13 million individuals in the U.S. are estimated to live in an area that exceeds this standard (Environmental Protection Agency, 2001).

Humans may be exposed to both organic and inorganic forms of arsenic. Exposure to organic arsenic compounds such as arsenobetaine, primarily via seafood ingestion, is generally considered to be minimally or non-toxic. The complex biotransformation of inorganic arsenic includes detoxification by subsequent methylation into monomethylarsonate (MMA) and dimethylarsenate (DMA) prior to excretion in the urine. Although all metabolites can be measured in the urine, DMA is most frequently detected in urine samples. Methylation is generally thought of as a detoxification process; however, some arsenical intermediates in the pathway (especially trivalent inorganic arsenic and MMA) are considered extremely toxic (Agency for Toxic Substances and Disease Registry, 2007). Urinary arsenic excretion has been accepted as an appropriate biomarker of internal dose as urinary arsenic levels are highly correlated to arsenic intake and reflect relatively

recent exposures (Centers for Disease Control and Prevention, Fourth Report on Human Exposures to Environmental Chemicals, 2009). Although some populations with high levels of arsenic exposure (e.g. Bangladesh) have shown some variability in urinary arsenic levels (Kile et al., 2009), a recent analysis of participants in the Strong Heart Study, a population-based study evaluating cardiovascular disease and risk factors in American Indian communities in Oklahoma, Arizona, and North and South Dakota, demonstrated long-term constancy of arsenic exposure and urinary excretion patterns, validating the use of urinary arsenic in large, population-based studies (Navas-Acien et al., 2009).

The kidney is the main organ involved in the excretion of arsenic and its metabolites, and thus several investigations have examined the effect of arsenic poisoning on kidney function. Many studies have examined albuminuria and/or proteinuria as the outcome of interest, as it is often an early marker of glomerular damage and may precede changes in GFR (Zheng et al., 2014). An occupational analysis of 127 male cobalt smelter workers exposed to arsenic trioxide showed an increased prevalence of proteinuria (Liu, 1989). An ecological study performed in southwestern Taiwan, an area of the world with endemic high exposure to arsenic, found a dose-response relationship between levels of inorganic arsenic exposure in well water and kidney cancer prevalence (Chen and Wang, 1990). Furthermore, kidney disease mortality in Taiwan decreased after the installation of public water supply systems and the reduction of arsenic in drinking water (Yang et al., 2004)

To date most investigations of the effects of arsenic on kidney function have been performed in populations with high levels of endemic arsenic exposure. Increasingly the adverse health effects of chronic exposure to low and moderate levels of arsenic (levels in drinking water from <10 to <50 μg/L) have been recognized (Naujokas et al., 2013). An ecological study conducted in southeastern Michigan (median water arsenic, 7.58 μg/L) found a positive association between moderate levels of water arsenic concentrations and kidney disease mortality (Meliker et al., 2007). A cross-sectional analysis of the Strong Heart Study recently found a dose-dependent effect of increasing urinary arsenic concentrations with prevalent albuminuria (Zheng et al., 2013). However, few epidemiological studies have directly examined associations of urinary arsenic with kidney function, and even fewer in young generally healthy populations with a low prevalence of chronic diseases such as diabetes and hypertension which are common causes of decreased kidney function and thus potent confounders. The objective of our study was to examine the association of urinary total arsenic and DMA with markers of kidney function in a representative population of young, generally healthy individuals with low to moderate arsenic exposure.

2. MATERIALS AND METHODS

2.1. Study Setting and Population

The National Health and Nutrition Examination Survey (NHANES) is a continuous nationally representative multistage random survey conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) designed to obtain a representative sample of the civilian noninstitutionalized U.S. population (CDC, 2014). We analyzed combined questionnaire, laboratory, and physical exam data from two survey cycles encompassing 2009 – 2012. The NHANES study protocols were approved by

the institutional review board of the National Center for Health Statistics. Oral and written informed consent was obtained from all participants, or if younger than 18 years, their guardians. Assent was obtained from all those aged 12 to 17 years. The participation rate in NHANES 2009–2010 was 77.3% and in 2011–2012 was 69.5%.

There were a total of 4,647 NHANES participants between the ages of 12 and 30 in the combined 2009–2012 NHANES study. Urinary arsenic measures were obtained on a one-third randomly selected subsample of NHANES participants (n=1,540) (CDC, 2011). Of the 1,540 eligible participants, 1,522 had urinary arsenic measures available. We excluded participants who were pregnant (n=19), missing serum creatinine (n=129), measured height (n=13), urine osmolality (n=56), body mass index (BMI) (n=3), or hypertension status (n=49), resulting in a final sample size of 1,253 participants.

2.2 Urinary Arsenic Measures

Urine samples were collected from participants at the time of the physical exam in arsenic-free containers and transported on dry ice to the Environmental Health Sciences Laboratory at the National Center for Environmental Health (NCEH: Atlanta, GA) (Caldwell et al., 2009). Urine samples were stored frozen at −70°C or lower and analyzed within 3 weeks of collection according to a standardized protocol (CDC, 2009). Total urinary arsenic concentrations were measured using inductively coupled-plasma dynamic reaction cell-mass spectrometry on a PerkinElmer ELAN 6100 DRC^{PLUS} or ELAN DRC II ICP-MS (PerkinElmer SCIEX, Concord ON, Canada) (CDC, 2011). Arsenic speciation can distinguish arsenic species that are directly related to inorganic arsenic exposure (arsenate, arsenite, MMA, and DMA) from organic arsenicals in seafood (arsenobetaine, arsenocholine) that are generally considered nontoxic (Fowler B.A. et al., 2007). All urinary arsenic species were measured using high performance liquid chromatography (HLPC).

Limits of detection (LOD) and interassay coefficient of variation (CV) differed among analytes and survey cycles. For total arsenic, the detection limit was 0.74 μg/L for the 2009–2010 survey and 1.25 μg/L for the 2011–2012 survey. For the 25 participants (2%) in whom total urinary arsenic levels were below the LOD, values were imputed by a level equal to the LOD divided by the square root of two (CDC, 2009). The LOD for the 2009–2010 and 2011–2012 cycle was 1.7 and 1.8 μg/L, respectively, for DMA; 0.9 and 0.89 μg/L for MMA; and 0.4 and 1.19 μg/L for arsenobetaine. For the 269 participants (21.6%) with DMA levels and 678 participants (54.4%) with arsenobetaine levels below the LOD their values were similarly assigned a level equal to the limit of detection divided by the square root of two. Although other arsenical metabolites such as arsenate, arsenite, and MMA were measured, they were excluded from this analysis due to the high percentage of undetectable levels (79.1%, 97.4%, and 68.4%, respectively). The interassay CV across NHANES lots ranged from 1.3% to 6.4% for total mean arsenic concentrations, 3.3% to 6.6% for DMA, and 5.3% to 7.3% for arsenobetaine.

2.3 Kidney Function Measures

Serum and urine creatinine levels were measured by the enzymatic method using a Roche/ Hitachi Modular P Chemistry Analyzer which is traceable to an isotope dilution mass

spectrometry (IDMS) reference standard. The interassay coefficients of variation ranged from 1.2% to 5.4% for serum creatinine (CDC, 2011) and 1.4 to 4.4 for urine creatinine (CDC, 2011). Urine osmolality was measured by the freezing point depression osmometry method. A primary function of the kidney is to filter waste products at the glomeruli and precisely regulate the excretion and absorption of nutrients and minerals (glucose, sodium, potassium, etc.) to maintain homeostatic balance. The most common indirect measure of kidney function is approximated by the collective filtration of all the glomeruli, referred to as GFR (measured in milliliters per minute per 1.73 m^2). As GFR is challenging and expensive to directly measure, we estimated GFR using the bedside GFR equation provided by the Chronic Kidney Disease in Children (CKiD) cohort study (0.413*height in centimeters/serum creatinine) (Schwartz et al., 2009) for participants 12–17 years of age, and the equation provided by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) for individuals 18 years or older (Levey et al., 2009).

2.4 Other Variables

Questionnaire information was analyzed to determine the participants' age, gender, and race/ethnicity. Physical examination provided BMI and blood pressure variables. The BMI was calculated as the weight in kilograms divided by the height in meters squared and percentiles were generated according to the CDC BMI-for-age gender-specific growth charts. Participants were categorized as overweight if their BMI was 90th – 94th percentile for participants ages 12–17, and BMI 25.0 to 29. 9 kg/m^2 for individuals 18 years. Participants were categorized as obese if their BMI was the 95th percentile or greater (ages 12–17) or 30 mg/m^2 (18 years). Participants between 12 and 17 years of age were classified as pre-hypertensive if they responded yes to a questionnaire or if their measured mean systolic or diastolic blood pressures were 90th – 94th percentile for age, height, and gender (National High Blood Pressure Education Program Working Group on High Blood Pressure in and Adolescents, 2004). Participants 18 years were classified as pre-hypertensive if they responded yes to a questionnaire or if their measured mean systolic blood pressure was between 120 and 139 mmHg or if their measured mean diastolic blood pressure was between 80 and 89 mmHg. Participants ages 12 to 17 were classified as hypertensive if they responded yes to a questionnaire or if their measured systolic or diastolic blood pressures were greater than the 95th percentile, (National High Blood Pressure Education Program Working Group on High Blood Pressure in and Adolescents, 2004) and participants 18 years were classified as hypertensive if they responded yes to a questionnaire or if their measured systolic blood pressure was 140 mmHg or greater or if their diastolic blood pressure was 90 mmHg or greater. Tobacco smoke exposure was determined based on serum cotinine levels measured by an isotope-dilution high performance liquid chromatography (HLPC) and atmospheric pressure chemical ionization tandem mass spectrometric method. Tobacco smoke exposure was categorized as undetectable (below the limit of detection, < 0.015 ng/mL), involuntary (0.015 – 10.0 ng/mL), and active (>10.0 ng/mL) (Benowitz, 1996). Diabetes was classified as yes if participants responded yes to a questionnaire that they had been diagnosed with diabetes by a doctor, or if the hemoglobin A1c was 6.5% or fasting glucose 126 mg/dL as per guidelines by the American Diabetes Association (American Diabetes, 2011). Albuminuria status was determined from random urine samples submitted by participants during the mobile exam center (MEC) component

of the survey. Albumin-to-creatinine ratios (ACR) were determined by dividing urinary albumin (mg/dL) by urinary creatinine (g/dL) and presented in categories ACR < 30 mg/g Cr, 30–300 mg/g Cr, and >300 mg/g Cr.

2.5 Statistical Analysis

All statistical analyses were performed using Stata statistical software, version 12.1 (StataCorp, College Station, Texas). Survey commands were utilized to account for the complex sampling design with appropriate strata, primary sampling units, and special sample weights used to obtained unbiased point estimates and robust standard errors (Johnson et al., 2013). The statistical significance level was set at alpha = 0.05, and all p-values were 2-sided.

Median and interquartile ranges (25th percentile and 75th percentile) for urinary total arsenic levels, DMA levels and eGFR were calculated for the entire study population. Urinary total arsenic is a reflection of both inorganic and organic arsenic. Because our question of interest was the relationship of inorganic arsenic with eGFR, our primary exposure assessment was (1) total urinary arsenical concentration and (2) DMA, adjusted for arsenobetaine. Natural log-transformed total arsenic and DMA were examined as continuous exposures and categorized as quartiles in multivariable linear regression models of eGFR. To account for differences in urinary concentration, we performed two parallel analyses by including adjustment for urinary creatinine or urine osmolality. First we adjusted for age, gender, and race/ethnicity. Further, we adjusted for potential chronic kidney disease risk factors including BMI, serum cotinine, hypertension status, and diabetes status. The final models were further adjusted for urinary arsenobetaine, which provides estimates for the association of exposure to inorganic arsenic and DMA independent of seafood ingestion.

Sensitivity analyses were performed with and without the covariates of poverty income ratio, diabetes, and hypertension. We also performed similar regression analyses accounting for urine dilution through the use of arsenic measures divided by the urine concentration correction factor (e.g., μg/g Cr or μg/μOsm/kg) as the exposure metrics. Secondary analyses were also performed using multiple linear regression techniques to examine the association of urinary arsenic species with ACR as the primary outcome variable. Similar regression models defining exposure to inorganic arsenic as the difference between total arsenic and arsenobetaine, as well as arsenobetaine characterized as a dichotomous variable (detectable versus non-detectable concentrations) were also explored. Normality assumptions of linear regression models were confirmed with visual plots of predicted values versus residuals, kernel density plots, and the Shapiro-Wilk test. Diagnostic tests for potential collinearity were performed by calculation of variance inflation factors (Liao and Valliant, 2012).

3. RESULTS

The median urinary total arsenic level in the 1,253 participants was 6.3 μg/L for total arsenic and 3.3 μg/L for DMA. The median eGFR was 109 mL/min/1.73 m^2 (Table 1). Total arsenic and DMA concentrations did not differ significantly by gender, tobacco exposure, BMI, hypertension status, or albuminuria status. White participants had lower total arsenic levels than non-white participants. DMA concentrations were higher in older participants. Total

arsenic and DMA were higher in participants without diabetes although very few participants had diabetes in this population (n=7). The eGFR was significantly higher in females, participants who were not black race, participants with diabetes, and participants without albuminuria. Total arsenic was modestly correlated with DMA (Pearson correlation coefficient r= 0.36) and highly correlated with arsenobetaine (r=0.98). Urinary creatinine was highly correlated with urine osmolality (r=0.72). Serum and urinary creatinine were modestly correlated (r=0.31). Serum creatinine and urine osmolality were not correlated (r=0.08).

Table 2 examines the association between urinary arsenic (total and DMA) and eGFR with urinary creatinine used to adjust for urine concentration. In the fully adjusted model, a log-unit increase in total arsenic was associated with a 4.0 mL/min/1.73 m^2 (95% CI 1.0 to 7.1 mL/min/1.73 m^2) higher eGFR, and a log-unit increase in DMA was associated with a 4.3 mL/min/1.73 m^2 (95% CI 0.5 to 8.0 mL/min/1.73 m^2) higher eGFR. Quartile analysis revealed a dose-dependent trend with participants in the highest quartiles of total arsenic or DMA having 10.1 mL/min/1.73 m^2 (95% CI 4.1 to 16.1 mL/min/1.73 m^2) and 10.7 mL/min/1.73 m^2 (95% CI 2.7 to 18.7 mL/min/1.73 m^2) higher eGFRs, respectively, as compared to the lowest quartile.

In contrast, analyses using urine osmolality identified a null association between arsenic or DMA and eGFR (Table 3). Log-unit increases of total arsenic and DMA were associated with a 0.4 mL/min/1.73 m^2 (95% CI −1.8 to 1.1 mL/min/1.73 m^2) and 0.01 mL/min/1.73 m^2 (95%CI −1.9 to 1.9 mL/min/1.73 m^2) lower eGFR, respectively. Comparing the highest to the lowest quartile, eGFR levels were 1.8 mL/min/1.73 m^2 (95% CI −2.2 to 5.8 mL/min/1.73 m^2) higher for total arsenic and 1.5 mL/min/1.73 m^2 (95% CI −2.3 to 5.2 mL/min/1.73 m^2) higher for DMA. Analyses examining the association between total arsenic or DMA and the difference in ACR (mg/g Cr) did not show any statistically significant differences.

We performed several sensitivity analyses. Inclusion of poverty income ratio in the models did not significantly change the results (data not shown). Exclusion of diabetes and hypertension also did not significantly change the results (data not shown), likely due to the low prevalence of diabetes and hypertension in this population. An analysis using the arsenic species divided by the urine concentration correction factor (e.g., μg/g Cr or μg/μOsm/kg) as the explanatory variable in the regression analyses showed results that were similar to our primary analysis. With creatinine adjustment, a log-unit increase in total arsenic was associated with a 6.7 mL/min/1.73 m^2 higher eGFR (95% CI 2.3 to 11.1 mL/min/1.73 m^2). With osmolality adjustment, the difference in eGFR was 1.0 mL/min/1.73 m^2 (95% CI −0.5 to 2.4 mL/min/1.73 m^2). Models defining inorganic arsenic as the difference between total arsenic and arsenobetaine showed similar results, although results did not achieve statistical significance (difference in eGFR of 1.4 mL/min/1.73 m^2, 95% CI −0.4 to 3.1 mL/min/1.73 m^2 with creatinine adjustment and −0.1 mL/min/1.73 m^2, 95% CI −1.3 to 1.0 mL/min/1.73 m^2 with osmolality adjustment). Finally, models adjusting for arsenobetaine as a dichotomous variable (detectable versus below LOD) showed similar results (difference in eGFR of 3.0 mL/min/1.73 m^2, 95% CI 0.3 to 5.7 mL/min/1.73 m^2 with creatinine adjustment and difference in eGFR of −0.5 mL/min/1.73 m^2, 95% CI −4.5 to 3.6 mL/min/1.73 m^2, with osmolality adjustment). Our data did not show evidence of

collinearity, as variance inflation factors for all regressions ranged below 1.1 and 2.7, markedly lower than 10, a cutoff commonly used to establish collinearity (Hair et al., 2009).

4. DISCUSSION

In this nationally representative sample of adolescents and young adults, associations of urinary total arsenic and DMA levels with eGFR were examined using two different methods of adjustment for urine concentration. Distinct differences were observed by method of adjustment. Higher total urinary arsenic and DMA levels, categorized as continuous or categorical variables, were associated with higher eGFR. However, these results were observed only when the arsenic measures were adjusted using urinary creatinine. Adjustment for urinary concentration using urine osmolality revealed null associations.

The understanding of human kidney processing of arsenic species continues to be poorly understood. Early experiments in dogs (Ginsburg, 1965) and more recent experiments using immortalized human proximal tubular epithelial cell lines (Peraza et al., 2003) have confirmed that the proximal tubular cell has a robust capacity to metabolize arsenic primarily by reducing arsenate to arsenite, and have identified cell mitochondria as a likely target for cell toxicity. Studies have demonstrated high activity levels of arsenite methyltransferase activity in the mouse kidney, indicating a potential role of the kidney in modifying susceptibility towards arsenic toxicity (Healy et al., 1998). Oxidative stress has been identified as a potential mechanism for arsenic-induced nephrotoxicity in mice, an effect that was partially reversed with the administration of arjunolic acid, a presumed free radical scavenger (Sinha et al., 2008). Human and animal studies have also shown increased lipid peroxidation, superoxide production, hydroxyl radical sulfhydrals, and/or oxidant-induced DNA damage resulting from arsenic exposure (Sasaki et al., 2007). In humans, acute arsenic intoxication has been associated with tubulointerstitial nephritis and acute tubular necrosis (Prasad and Rossi, 1995).

Although there are relatively few epidemiological studies examining low level arsenic exposure and kidney function, our findings of a positive association between urinary arsenic and GFR differ from most previous publications. A case-control study examining 125 patients with chronic kidney disease (CKD) and 229 age-matched controls (mean eGFR 28 and 80 mL/min/1.73 m^2, respectively) showed that total urinary arsenic level was significantly associated with CKD in a dose-response relationship, with those in the highest tertile of total arsenic having a more than four-fold increase in odds of CKD versus controls (Hsueh et al., 2009). Estimated GFR was also significantly negatively associated with total urinary arsenic levels in another study of similar design comparing 132 patients with renal cell carcinoma (mean arsenic 25.2 μg/L) to age- and sex- matched controls (mean arsenic 21.1 μg/L) (Huang et al., 2011). Both of these studies accounted for urine concentration by correction with urine creatinine (i.e., exposure categorized as μg/g creatinine). However, a recent study in the Strong Heart cohort reported that the sum of inorganic and methylated arsenic species was inversely related to prevalent CKD and thus, similar to the positive associations we observed (Zheng et al., in press). Interestingly, arsenic was associated with increased risk for incident CKD.

To our knowledge there are no prior published studies examining the association between arsenic species and eGFR with direct comparison of creatinine and non-creatinine-based methods of adjustment for urine concentration. The reason for the unexpected positive association between eGFR and total urinary arsenic observed with adjustment by urinary creatinine remains unclear. Similar unexpected positive associations between suspected nephrotoxicants, including lead, cadmium, and thallium, and kidney function outcomes have been described in the literature, particularly when using serum creatinine-based eGFR measures and urinary creatinine to adjust for urinary dilution (de Burbure et al., 2006; Ferraro et al., 2010; Shelley et al., 2012; You et al., 2011). One potential explanation in our cross-sectional study could be that the higher levels of urinary arsenic simply reflect higher kidney filtration. A wide spectrum of "normal" GFR exists due to a variety of biological factors and nephron endowment, and normal GFR ranges from approximately 90–130 mL/min/1.73 m^2 (Schwartz and Work, 2009). This potential explanation would be related to reverse causality and could have substantial impact on any environmental epidemiological research which utilizes urine samples to assess toxicant exposure, as researchers would potentially need to account for the effect of kidney filtration on the toxicant level in the urine when analyzing and interpreting results. Urinary levels have been accepted as surrogate markers for cumulative exposure or internal dose for many metals, including inorganic mercury, cadmium, and arsenic (Roels et al., 1999). Another plausible explanation is the glomerular hyperfiltration theory. Hyperfiltration is a complex phenomenon that has been described in multiple pathophysiological states including diabetes, sickle cell disease, and obesity. It is characterized by maladaptive alterations in glomerular hemodynamics and structural changes within the glomerulus and is often associated with albuminuria and a transient *increase* in GFR (Helal et al., 2012). Although hyperfiltration has been proposed in an experimental animal model which demonstrated a positive association between blood lead and measured GFR (Khalil-Manesh et al., 1992), hyperfiltration is unlikely to be the cause for the results observed herein given that there were no significant associations observed between increased arsenic levels and albuminuria. Additionally, if the physiological filtration or pathological hyperfiltration theories were true, one would also expect a positive association with the osmolality adjusted analyses, which was not observed. The potential for alterations in proximal tubular secretion of creatinine which may be induced by arsenic, also known to affect the proximal tubule, is a mechanism which cannot be excluded. This possibility was highlighted in a recent study (Weaver et al., 2014) which also demonstrated unexpected positive associations between urine cadmium, thallium, and uranium and estimated GFR only when urine creatinine, not urine osmolality, was used to adjust for urine concentration.

Another potential explanation for the inconsistent findings between urinary creatinine and urine osmolality could be due to residual confounding from inadequate adjustment for urinary concentration. It is well-known that urinary biomarkers are highly dependent on urine concentration, and several studies have shown considerable variation in biomarkers relative to time of day of the urine collection (Akerstrom et al., 2014). For example, concentrations tend to be higher in first-morning voids and lower in more dilute spot urine samples collected later in the day, presumably due to variations in hydration status. In principle, this source of variation should be non-differential and bias the associations

towards the null, although prior work by Christensen et al. examining urinary phthalate has demonstrated that adjustment with urine creatinine biased the outcome of interest away from the null (Christensen et al., 2014). Several approaches have been proposed to account for this important source of variation, although no consensus exists on the optimal methodological approach to correction for urine concentration (Barr et al., 2005). The most commonly employed method in arsenic research divides the arsenic concentration by urinary creatinine, and results are presented as ug/g creatinine. However, there are well-described limitations with the use of creatinine which include important variations relative to gender, age, muscle mass, and diet, as well as substantial processing in the kidney including tubular secretion and filtration which varies significantly by level of kidney function (Gamble and Hall, 2012; Perrone et al., 1992; Waikar et al., 2010). Alternative methods to account for urine concentration have been explored, including adjustment for specific gravity, osmolality, and using timed urine collections (Akerstrom et al., 2012; Suwazono et al., 2005). Urine osmolality is rarely used to adjust for urine concentration, despite the fact that it is less dependent on large molecular weight molecules (e.g. glucose or albumin) in the urine in contrast to urine specific gravity, and may be more useful in children and adolescents in whom creatinine varies greatly by age (Chadha et al., 2001; Yeh et al., 2015).

Conflicting associations depending on approach to the urine concentration were highlighted in a recent investigation which examined the association of urinary uranium with several eGFR and creatinine clearance outcomes in a population of Korean lead workers environmentally exposed to uranium (Shelley et al., 2014). The authors directly compared three different approaches, including directly dividing uranium level by urinary creatinine, including urinary creatinine as a covariate with uranium in the regression model, and the use of a timed specimen in which the total amount of uranium excreted over 4 hours was calculated. Statistically significant negative associations were observed with measured creatinine clearance in the models that used urinary creatinine but total excreted uranium was not significantly associated with any outcomes. The authors concluded that the observed associations between urinary toxicants and kidney outcomes may be related to statistical associations due to the use of urinary creatinine in both exposure and outcome metrics. We cannot rule out the possibility that the positive association in our study may also be partially statistical in nature, due to the modest positive correlation between serum and urine creatinine.

One major limitation of our investigation is inherent to the cross-sectional design which limits the ability to draw causal inference between urinary arsenic and eGFR. These cross-sectional findings could reflect reverse causality. A second limitation is the lack of measured GFR in this population and our reliance on estimating equations, which have well-known limitations, particularly in the adolescent population (Schwartz and Work, 2009). In particular, none of the GFR estimating equations, including the two used in our study, have been validated in the 17–19 year old age group. A third limitation is the lack of a timed urine collection, which although expensive and challenging to obtain would allow for a comparison of results by method of urine concentration adjustment to a "gold standard" assessment of arsenic excretion. Lastly, we did not have information on arsenic levels in food or water, the primary sources of arsenic exposure in general populations. However,

urinary arsenic is considered a valid biomarker that integrates exposure from multiple sources (Zheng et al., 2014).

Despite these limitations, to our knowledge this is the first study to examine the association of urinary arsenic and arsenic species on eGFR in a young and healthy population with low to moderate levels of arsenic exposure. This is also one of the first studies to demonstrate that method of adjustment for urinary concentration impacts the observed associations with arsenic exposure. The implications for future arsenic research, and potentially other toxicants for which urinary levels are used to assess exposure, could be substantial. Given the growing body of evidence that shows conflicting and unexpected associations with kidney outcomes when adjustment methods employ the use of urinary creatinine, future study should be dedicated to validating the best approach to account for urinary concentration. This study also adds further insight into the understanding of the potential epidemiological associations between arsenic and kidney function and highlights the need to better understand the health consequences for populations with chronic exposure to low to moderate levels of arsenic.

Acknowledgments

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Highlights

- A cross-sectional study of arsenic and markers of kidney function is presented.

- positive association is shown with adjustment for urinary creatinine.

- No association exists with adjustment for urine osmolality.

- More research on the best adjustment method for urine concentration is needed.

Table 1

Total Urinary Arsenic (ug/L)[a], DMA (ug/L) and eGFR by Participant Characteristics[b]

Characteristic	No (%) of participant[c]	Total arsenic (μg/L)	p-value[d]	DMA (μg/L)	p-value[d]	eGFR (mL/min/1.73m²[e])	p-value[d]
Total	1,253 (100)	6.3 (3.3, 12.7)	---	3.3 (1.7, 5.7)	---	109 (95, 122)	---
Sex:							
Male	673 (53.7)	6.7 (3.7, 15.3)	0.34	3.6 (1.9, 6.2)	0.12	106 (93, 120)	0.008
Female	580 (36.3)	5.7 (2.8, 11.0)		2.9 (1.3, 5.2)		112 (97, 124)	
Age (years):							
12–17	543 (33.8)	5.4 (3.0, 9.9)	0.52	3.1 (1.8, 4.9)	0.025	99 (88, 110)	0.051
18–24	464 (40.2)	6.2 (3.5, 12.6)		3.3 (1.8, 5.8)		115 (103, 126)	
25–30	246 (26.1)	8.4 (3.4, 19.7)		3.6 (1.3, 7.0)		112 (98, 121)	
Race/Ethnicity:							
Black	301 (13.5)	6.8 (4.1, 14.0)	0.021	3.6 (2.1, 6.3)	0.44	98 (87, 114)	<0.001
Hispanic	378 (20.1)	7.8 (4.3, 13.2)		4.0 (2.3, 6.5)		118 (103, 127)	
White	404 (57.7)	5.3 (3.0, 11.4)		2.8 (1.3, 4.8)		108 (96, 120)	
Other	170 (8.7)	7.6 (3.8, 29.7)		3.8 (2.1, 10.2)		111 (95, 125)	
Tobacco exposure:[f]							
Undetectable	262 (22.9)	5.6 (2.8, 12.6)	0.24	3.3 (1.3, 6.1)	0.48	111 (96.4, 122)	0.95
Involuntary	748 (57.0)	6.1 (3.2, 13.2)		3.2 (1.8, 5.5)		107 (94, 122)	
Active	243 (20.0)	7.3 (4.3, 14.5)		3.6 (2.0, 6.7)		112 (97, 122)	
Weight category:[g]							
Underweight	44 (2.9)	6.2 (3.6, 11.9)	0.31	3.3 (1.3, 4.9)	0.54	121 (104, 129)	0.51
Normal weight	641 (52.4)	6.1 (3.4, 12.5)		3.2 (1.7, 5.5)		108 (95, 120)	
Overweight	266 (21.9)	6.2 (3.1, 11.4)		3.2 (1.7, 5.1)		109 (95, 122)	
Obese	302 (22.7)	7.2 (3.2, 14.9)		3.8 (1.9, 7.2)		111 (97, 123)	
Hypertension:[h]							
No hypertension	1,022 (78.7)	6.2 (3.3, 11.8)	0.35	3.2 (1.8, 5.6)	0.83	109 (95, 122)	0.82
Pre-hypertension	209 (19.4)	7.3 (3.0, 19.7)		3.4 (1.7, 6.8)		111 (97, 121)	
Hypertension	22 (1.9)	7.4 (3.4, 14.9)		3.3 (2.0, 4.1)		111 (90, 121)	
Diabetes:[i]							
No	1,246 (99.4)	6.4 (3.3, 12.7)	<0.001	3.3 (1.8, 5.7)	0.04	109 (95, 122)	0.004

Characteristic	No (%) of participant[c]	Total arsenic (µg/L)	p-value[d]	DMA (µg/L)	p-value[d]	eGFR (mL/min/1.73m^2[e]	p-value[d]
Yes	7 (0.6%)	2.7 (0.9, 6.9)		1.3 (1.3, 5.4)		136 (125, 136)	
ACR (mg/g Cr):							
<30	1,150 (92.0)	6.3 (3.3, 12.7)	0.20	3.2 (1.7, 5.8)	0.89	109 (96, 122)	0.039
30–300	98 (7.6)	6.5 (3.2, 15.4)		3.1 (2.0, 5.1)		104 (92, 115)	
>300	5 (0.4)	8.0 (2.4, 13.8)		4.5 (1.3, 4.5)		100 (97, 115)	

[a]For SI conversions from µg/L to µmol/L, multiply by 0.0133.

[b]Data are given as weighted median (interquartile range).

[c]Weighted percentage.

[d]P-values were obtained based on linear regression models comparing arsenic levels by participant characteristics.

[e]GFR based on bedside CKiD equation for participants ages 12 to 17 years, and by the equation provided by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) for participants ages 18 years and above.

[f]Undetectable: serum cotinine <0.015 ng/mL; Involuntary: 0.015 – 10.0 ng/mL; Active: >10.0 ng/mL

[g]Underweight: BMI <5th percentile <(12–17 years) or BMI <18.5 kg/m^2 (≥18 years); Normal weight: BMI 5th to 89th percentile <(12–17 years) or BMI 18.5 to 24.99 kg/m^2 (≥18 years); Overweight: BMI 90th to 94th percentile <(12–17 years) or BMI 25 to 29.99 kg/m^2 (≥18 years); Obese: BMI ≥95 percentile (12–17 years) or BMI ≥30 kg/m^2 (≥18 years).

[h]Pre-hypertension: Self-report or SBP or DBP 90–94th percentile (12–17 years) or SBP 120–139 mmHg or DBP 80–89 mmHg (≥18 years); Hypertension: self-report or SBP or DBP ≥95 percentile (12–17 years) or SBP ≥140 mmHg or DBP ≥90 mmHg.

[i]Self-report, fasting blood sugar > 126 mg/dL, or hemoglobin A1c ≥6.5%.

Table 2

Difference in eGFR Associated with Urinary Total Arsenic and DMA Levels; Adjusted for Urinary Creatinine[a]

Characteristic	N	Unadjusted	Model 1[b]	Model 2[c]	Model 3[d]
Total Arsenic[e]	1,253	−1.3 (−2.8, 0.2)	1.03 (−0.8, 2.8)	1.2 (−0.5, 2.9)	4.0 (1.0, 7.1)
Arsenic quartile					
1 (<3.6 µg/L)	315	0 (ref)	0 (ref)	0 (ref)	0 (ref)
2 (3.6 – 6.8 µg/L)	313	−1.2 (−6.1, 3.7)	4.5 (0.4, 8.5)	4.5 (0.5, 8.4)	5.2 (1.3, 9.1)
3: (6.9 – 13.1 µg/L)	311	−3.4 (−7.1, 0.3)	3.0 (−1.8, 7.9)	3.3 (−1.7, 8.3)	5.0 (−0.3, 10.3)
4: (> 13.1 µg/L)	314	−2.8 (−7.0, 1.5)	5.0 (0.02, 9.9)	5.4 (0.4, 10.3)	10.1 (4.1, 16.1)
p-value for trend		0.118	0.141	0.103	0.012
DMA[e]	1,253	−1.7 (−3.6, 0.02)	2.9 (−0.5, 6.3)	3.0 (−0.2, 6.3)	4.3 (0.5, 8.0)
DMA quartile					
1 (< 1.9 µg/L)	316	0 (ref)	0 (ref)	0 (ref)	0 (ref)
2 (1.9 – 3.5 µg/L)	312	−1.3 (−5.5, 2.9)	5.5 (0.4, 10.7)	5.5 (−0.5, 10.5)	6.2 (1.0, 11.3)
3 (3.6 – 6.0 µg/L)	312	−6.5 (−10.5, −2.5)	3.0 (−2.8, 8.9)	3.3 (−2.3, 8.9)	4.4 (−1.4, 10.2)
4 (>6.0 µg/L)	313	−2.4 (−6.0, 1.1)	8.4 (0.7, 16.2)	8.7 (1.3, 16.1)	10.7 (2.7, 18.7)
p-value for trend		0.035	0.069	0.046	0.023

[a] Data provided as the difference in eGFR in milliliters per minute per 1.73 m^2 (95% confidence interval) with arsenic species modeled continuously (per log unit increase) and as quartiles (lowest quartile is reference category).

[b] Model 1 is adjusted for age, gender, race/ethnicity, and log urinary creatinine.

[c] Model 2 is further adjusted for BMI, log serum cotinine, hypertension, and diabetes.

[d] Model 3 is further adjusted for log urinary arsenobetaine.

[e] Arsenic, DMA log-transformed for linear regression analysis.

Table 3

Difference in eGFR Associated with Urinary Total Arsenic and DMA Levels; Adjusted for Urine Osmolality [a]

Characteristic	N	Unadjusted	Model 1[b]	Model 2[c]	Model 3[d]
Total Arsenic[e]	1,253	−1.3 (−2.8, 0.2)	−0.6 (−2.0, 0.9)	−0.5 (−1.9, 1.0)	−0.4 (−1.8, 1.1)
Arsenic quartile					
1 (<3.6 µg/L)	315	0 (ref)	0 (ref)	0 (ref)	0 (ref)
2 (3.6 – 6.8 µg/L)	313	−1.2 (−6.1, 3.7)	1.2 (−3.2, 5.6)	1.0 (−3.3, 5.3)	1.2 (−3.1, 5.5)
3: (6.9 – 13.1 µg/L)	311	−3.4 (−7.1, 0.3)	−1.9 (−5.6, 1.8)	−1.8 (−5.5, 1.9)	−1.3 (−4.7, 2.1)
4: (> 13.1 µg/L)	314	−2.8 (−7.0, 1.5)	−0.1 (−4.4, 4.4)	0.1 (−4.4, 4.5)	1.8 (−2.2, 5.8)
p-value for trend		0.118	0.68	0.732	0.738
DMA[e]	1,253	−1.7 (−3.6, 0.02)	−0.3 (−2.5, 1.9)	−0.3 (−2.4, 1.8)	−0.01 (−1.9, 1.9)
DMA quartile					
1 (< 1.9 µg/L)	316	0 (ref)	0 (ref)	0 (ref)	0 (ref)
2 (1.9 – 3.5 µg/L)	312	−1.3 (−5.5, 2.9)	0.6 (−3.4, 4.6)	0.6 (−3.2, 4.4)	0.8 (−2.9, 4.6)
3 (3.6 – 6.0 µg/L)	312	−6.5 (−10.5, −2.5)	−3.6 (−7.3, 0.2)	−3.5 (−7.0, 0.01)	−3.1 (−6.3, 0.1)
4 (>6.0 µg/L)	313	−2.4 (−6.0, 1.1)	0.6 (−4.0, 5.3)	0.6 (−3.8, 5.0)	1.5 (−2.3, 5.2)
p-value for trend		0.035	0.824	0.817	0.973

[a] Data provided as the difference in eGFR in milliliters per minute per 1.73 m^2 (95% confidence interval) with arsenic species modeled continuously (per log unit increase) and as quartiles (lowest quartile is reference category).

[b] Model 1 is adjusted for age, gender, race/ethnicity, and urine osmolality.

[c] Model 2 is further adjusted for BMI, log serum cotinine, hypertension, and diabetes.

[d] Model 3 is further adjusted for log urinary arsenobetaine.

[e] Arsenic, DMA log-transformed for linear regression analysis