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Urinary arsenic species, toenail arsenic, and arsenic intake estimates in a Michigan population with low levels of arsenic in drinking water

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Abstract

The large disparity between arsenic concentrations in drinking water and urine remains unexplained. This study aims to evaluate predictors of urinary arsenic in a population exposed to low concentrations (≤50 μg/l) of arsenic in drinking water. Urine and drinking water samples were collected from a subsample (n = 343) of a population enrolled in a bladder cancer case–control study in southeastern Michigan. Total arsenic in water and arsenic species in urine were determined using ICP-MS: arsenobetaine (AsB), arsenite (As[III]), arsenate (As[V]), methylarsenic acid (MMA[V]), and dimethylarsenic acid (DMA[V]). The sum of As[III], As[V], MMA[V], and DMA[V] was denoted as SumAs. Dietary information was obtained through a self-reported food intake questionnaire. Log_{10}-transformed drinking water arsenic concentration at home was a significant (P<0.0001) predictor of SumAs (R^2 = 0.18). Associations improved (R^2 = 0.29, P<0.0001) when individuals with less than 1 μg/l of arsenic in drinking water were removed and further improved when analyses were applied to individuals who consumed amounts of home drinking water above the median volume (R^2 = 0.40, P<0.0001). A separate analysis indicated that AsB and DMA[V] were significantly correlated with fish and shellfish consumption, which may suggest that seafood intake influences DMA[V] excretion. The Spearman correlation between arsenic concentration in toenails and SumAs was 0.36 and between arsenic concentration in toenails and arsenic concentration in water was 0.42. Results show that arsenic exposure from drinking water consumption is an important determinant of urinary arsenic concentrations, even in a population exposed to relatively low levels of arsenic in drinking water, and suggest that seafood intake may influence urinary DMA[V] concentrations.

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Conflict of interest

The authors declare no conflict of interest.

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Keywords

arsenic exposure; arsenic metabolites; categorical variables; exposure metric; arsenobetaine; organoarsenicals

Introduction

Urinary excretion of arsenic is the primary pathway for the elimination of arsenic from the human body (Le et al., 1994). In addition, the collection of urine samples is a non-invasive procedure, and hence is an attractive methodology for large-scale studies. Epidemiological studies have shown positive and consistent relationships between arsenic in drinking water and arsenic in urine. For example, in Taiwan, Hsueh et al. (1997) found that people with high arsenic levels in their drinking water have higher concentrations of arsenic and its metabolites in urine than people exposed to lower levels of arsenic in water. After termination of arsenic exposure through drinking water, populations show a decrease in levels of urinary arsenic metabolites (Tseng et al., 2005). A study of cancer risk in Hungary, Romania, and Slovakia ($n = 537$) showed a significant correlation between arsenic in drinking water and the sum of arsenic species (As[III] (arsenite), As[V] (arsenate), MMA[V] (methylarsenic acid), DMA[V] (dimethylarsenic acid)) in urine ($R^2 = 0.45$, $P<0.0001$) (Lindberg et al., 2006).

An increasing awareness of the potential chronic health effects of arsenic at low exposure levels has motivated efforts to better understand biomarkers of exposure. In a New Hampshire population where 99% of the individuals had arsenic concentrations less than 50 μg/l in their drinking water, Karagas et al. (2001) found a correlation of 0.35 ($P = 0.0024$) between arsenic concentrations in water and in urine, and the association increased to 0.46 ($P = 0.029$) when they excluded drinking water with less than 1 μg/l of arsenic (Karagas et al., 2002). In addition to arsenic concentration alone, available evidence suggests that volume of water intake may be an influential modifier of ingestion exposures (Wright et al., 2006). A study conducted in Mexico where the population was exposed to arsenic in drinking water between 3.3 and 49.3 μg/l showed that considering total arsenic intake per day influenced the correlation between arsenic concentrations in water and urinary arsenic (Meza et al., 2004). The correlation coefficient improved from 0.35 ($P = 0.02$) to 0.50 ($P<0.0001$) when the volume of water intake was included in the exposure metric. This finding suggests that incorporating water consumption may improve prediction of arsenic in urine. Likewise, intake of water, beverages or foods made with tap water, or the consumption of other foods, such as fish, also influence arsenic exposure (Xue et al., 2010) and the amount of arsenic excreted in urine.

Previous studies have also evaluated the relationship between arsenic levels measured in urine and toenails. Karagas et al. (2001) found a correlation of 0.36 ($P = 0.0012$) between urinary arsenic and toenails in 77 individuals. A study in Nevada analyzed arsenic in toenails and total arsenic in urine in 95 individuals (Adair et al., 2006). Toenails were analyzed using two different methods, neutron activation analysis (NAA) and hydride generation-atomic

fluorescence spectroscopy. Only samples analyzed with NAA were significantly correlated with arsenic concentration in urine ($r = 0.30$, $P<0.0001$).

A primary strength that urine offers as a biomarker for arsenic exposure is the ability to characterize arsenic species or metabolites. Organic species derived primarily from seafood such as arsenobetaine (AsB) or arsenosugars can be identified, as can the contribution of toxic species. The metabolism of inorganic arsenic in humans results in methylarsonate (MMA) and dimethylarsinate (DMA), which are excreted together in urine (Aposhian and Aposhian, 2006). Organoarsenicals are also excreted in urine, but they have low or no toxicity compared with inorganic arsenic (Francesconi and Kuehnelt, 2004). How seafood consumption and organoarsenicals influence inorganic arsenic excretion is not completely known. It has been suggested that seafood consumption not only increases the excretion of organoarsenicals, but DMA as well (Francesconi et al., 2002). Navas-Acien et al. (2011) reported a 1.4-fold (95% confidence interval 1.2, 1.6) higher concentration of DMA in individuals reporting seafood intake ≥2 times per week compared to never during the past year using 2003–2006 National Health and Nutrition Examination Survey (NHANES). DMA concentrations were also higher ($P = 0.01$) in individuals reporting consumption of seafood in the 3 days before urine collection in residents living in an area with soil naturally rich in arsenic in France (Fillol et al., 2010). The sum of inorganic and methylated arsenic species is commonly used as a biomarker of inorganic arsenic exposure. However, if AsB and seafood consumption can increase DMA in urine, a better characterization of DMA is essential for evaluating the relationship between urinary arsenic and arsenic exposure.

In an attempt to better characterize exposure biomarkers and reduce exposure misclassification in epidemiological studies, it is critical to correctly identify individual sources of exposure through water consumption or other pathways (Maskiell et al., 2006). We investigated a population exposed to low levels of arsenic (≤50 μg/l) in southeastern Michigan. The primary aim of this study was to evaluate predictors of urinary arsenic concentrations. A secondary aim was to compare exposure metrics such as water intake and food to identify factors that impact urinary arsenic excretion. In addition, separate analyses were performed to evaluate the relationship between seafood intake and urinary AsB and DMA [V]. Finally, a third aim of the study was to compare arsenic in water, arsenic in urine, and arsenic in toenails as markers of arsenic exposure in our population.

Materials and methods

Study Subjects

Spot urine samples were collected from a subsample of participants in southeastern Michigan enrolled in a case–control study of arsenic exposure and bladder cancer. Cases were obtained from the Michigan State Cancer Registry and were frequency-matched by age, race, and gender, with controls selected through a random-digit dialing procedure. Cases as well as controls were recruited on the basis that they had been living in the study area for at least 5 consecutive years before recruitment. The subsample used in this study included 151 cases and 192 controls recruited from June 2005 through May 2007. Invitations were extended to this subsample based on their likelihood of drinking groundwater, the main source of drinking water arsenic in this population. As evidence of

this recruitment strategy, individuals in this subsample had a slightly higher concentration of drinking water arsenic (5.1 μg/l) (arithmetic mean) than the main sample (3.5 μg/l) (arithmetic mean). All of those individuals in the case–control study who were invited to participate in this substudy agreed to participate. Each participant signed an informed consent form and random identification numbers were assigned to each participant to maintain confidentiality. Further details on the selection of subjects are described elsewhere (Meliker et al., 2010).

Interview Data and Food Intake Questionnaire

Phone interviews to obtain demographic information, medical history, lifestyle habits, and water consumption patterns of individuals were conducted (Slotnick et al., 2007). For the purpose of this study, exposure data were obtained from a Food Intake Questionnaire (FIQ) derived from instruments used in previous studies of arsenic exposure in the United States (Karagas et al., 2004; Steinmaus et al., 2005). The questionnaire was limited to the 3 days before the urine sample collection. The questionnaire was administered during personal home interviews where information on consumption of specific food items, home tap water, vitamins, and smoking and drinking habits were obtained. Food items included rice, chicken, mushrooms, fish (e.g., cod, salmon, haddock, trout), shellfish (e.g., shrimp, lobster, clams), and sushi among others. Specifically, participants were asked how many servings of each food item they had consumed in the past 3 days. Likewise, they were asked about home tap water consumption per day: plain tap water at home and beverages made with tap water at home (e.g., coffee, hot or iced tea).

From all the different food items included in the FIQ, only those with at least 10% response were included in the final analysis. These foods included rice (10%), mushrooms (29%), and chicken (28%). Individual kinds of fish (haddock, salmon, cod), shellfish (shrimp, clams, lobster), sushi, or seaweed were not reported by at least 10% of the respondents and were therefore combined under ‘‘any seafood.’’ Rice, mushrooms, chicken, and seafood were evaluated as single variables and as part of exposure Metric 4 described below. Although fish and shellfish were reported by less than 10% of the population, they were included in the individual analysis after exploratory analyses showed that ‘‘any seafood’’ was associated with SumAs. Food intake data were compiled using serving sizes to estimate how much of a food item a participant ate during any of the 3 days requested in the questionnaire. The serving amounts were provided in ounces. Serving sizes were converted to the metric system and multiplied by the amount of arsenic in foods reported in the literature. Analyses were conducted for each of the 3 days for water and food intakes. There were no differences in the results of individual days or including all 3 days together; therefore, results are presented for all 3 days together.

Collection and Analysis of Water Samples

During home visits, tap water samples were collected. Participants identified their main drinking water sources and additional sources that they used for cooking and coffee, if different. Water samples were collected in low-density polyethylene bottles acid-washed for determination of trace metals. Samples were immediately stored on ice until arriving at the

laboratory where they were acidified with 100 μg/l trace-metal grade nitric acid (Fisher Chemical, Pittsburgh, PA, USA) and stored until analysis.

All samples were analyzed at the University of Michigan, School of Public Health using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent Technologies Model 7500c). National Institute of Standards and Technology Standard Reference Materials (SRM) 1640, Trace Elements in Natural Water, was used to validate the calibration. The SRM 1640 was always within 10% of the certified value for arsenic. The arsenic detection limit for water was three times the standard deviation of the calibration standard diluted to 0.05 μg/l. The minimum detection level (MDL) for this set of samples was calculated as 0.046 μg/l. Samples below the detection limit were set to the limit of detection divided by the square root of 2. Additional details on water collection and analysis have been published elsewhere (Slotnick et al., 2007).

Collection and Analysis of Toenail Samples

Specific methods on collection, preparation, and analysis of toenail samples in this population have been published previously (Slotnick et al., 2007). Briefly, clipping materials and instructions were mailed to participants after enrollment into the study. Participants were asked to clip all 10 toenails after bathing or showering, and record the date of clipping and time since their last clipping. After collection, samples were washed and digested following modified protocols (Das et al., 1995; Karagas et al., 2000). Toenails were analyzed for arsenic using ICP-MS, and toenail MDL for this set of samples was 0.043 μg/g ($n = 7$).

Collection and Analysis of Urine Samples

The research team provided participants with materials and instructions for urine collection. Spot samples were immediately frozen in dry ice brought along by the research team and then transported to the laboratory. Spot samples were stored in the laboratory at −20 °C until analysis. Urine samples were filtered through a 5 μm 250 × 4.6 mm^2 column (Phenomenex, Torrance, CA, USA). The mobile phase contained 4% (v/v) methanol, 5 mM tetrabutylammonium hydroxide, and 10 mM ammonium phosphate at pH 9.5. The high-performance liquid chromatography system (Alltech, Lexington, KY, USA) was coupled to the ICP-MS unit. Detection limits for arsenic species by the method used were as follows: AsB, 0.06 μg/l; As[III], 0.112 μg/l; As[V], 0.147 μg/l; MMA[V], 0.117 μg/l; and DMA[V], 0.076 μg/l. Urine certified reference materials from the Japanese National Institute for Environmental Studies (NIES No. 18, Tsukuba, Ibaraki, Japan) were used as reference standards. Concentrations were adjusted to the mean specific gravity of the samples (1.018 g/ml). Samples below the detection limit were set to the limit of detection divided by the square root of 2. The percent of samples below detection limit were 46.6%, 16.9%, 0.29%, and 0.58% for inorganic arsenic (As[III] and As[V]), MMA[V], DMA[V], and AsB, respectively. Details of the analytical protocol (Rivera-Núñez et al., in press) and sample collection have been described previously (Rivera-Núñez et al., 2010).

Calculation of Exposure Metrics

Exposure metrics for each participant were calculated using arsenic concentration in drinking water, drinking water intake, and FIQ data. Four different metrics were developed

as potential predictors of arsenic exposure: (Metric 1) arsenic concentration in drinking water at home (μg/l); (Metric 2) arsenic intake (μg per day) from plain drinking tap water at home; (Metric 3) arsenic intake (μg per day) from tap water and beverages made with tap water at home; and (Metric 4) arsenic intake (μg per day) from food (inorganic arsenic only) and all tap water sources at home (Supplementary Table S1). Water intake in Metrics 2 and 3 was calculated by multiplying water consumption (l per day) by respective arsenic water concentrations (μg/l). Estimated intake (μg/l) in Metric 4 was calculated by multiplying food consumption (g per day) by inorganic arsenic concentrations (μg/g). Inorganic arsenic in different foods was estimated by averaging across the literature (Supplementary Table S2). Comparable methods have been employed (Meacher et al., 2002) and used in our previous reports (Slotnick et al., 2007). Frequency and quantity (serving size) was obtained from the FIQ.

Statistical Analysis

Descriptive statistics were calculated for arsenic concentrations in drinking water, toenails, and urine. Histograms and normal probability plots revealed deviations from a normal distribution for water arsenic concentrations, toenail arsenic concentrations, and all urinary arsenic metabolites. Log_{10} transformations were applied to the data before performing statistical analyses. The sum of As[III], As[V], MMA[V], and DMA[V] was log_{10}-transformed and used to designate sum of inorganic and methylated species (SumAs). AsB was not included in SumAs to distinguish between the toxic inorganic arsenic compounds and the less toxic AsB. When AsB was included in the log_{10}-transformed sum, it was designated as total arsenic (TotAs). The sum of As [III] and As[V] was used as the total of inorganic arsenic (InAs) because it provides a more stable measure of inorganic arsenic InAs in urine, as these two species may interconvert while in urine. As MMA[III] and DMA[III] were not detected in any of the samples, MMA and DMA will refer only to the respective pentavalent species, unless otherwise stated.

The association of urinary arsenic with demographic variables such as disease status, gender, age, and smoking was assessed using bivariate analyses. Student's *t*-test was used to compare category means. One-way analysis of variance was used when three or more means were compared. A smoker was defined as a person who reported at least one cigarette per day in the FIQ. Age, gender, smoking, and body mass index (BMI) were evaluated as single variables as well as in multiple regression models to assess confounding.

Spearman's correlation (r_s) procedures were applied to investigate the relationship between urinary arsenic concentration, water arsenic concentrations, and toenail arsenic concentration. Linear regression models were used to evaluate the ability of exposure metrics and other variables to predict arsenic concentrations in urine. Log-transformed urinary arsenic concentration (SumAs) was modeled as a dependent variable against Metrics 1, 2, 3, and 4. There were no differences in the analyses when including individual food items as different metrics or categorical variables; all foods were considered together as a continuous variable in Metric 4. Treating food consumption categorically (yes/no), defined by eating a single item more than one time in the 3 days before the urine sample collection also did not influence the results. Nonetheless, to evaluate food as a predictor in separate

analyses (not metrics), individual food items (e.g., chicken, mushrooms, canned fish) were used in the models to predict SumAs. Individual food items were also used to predict urinary AsB. Spearman's correlation coefficients were produced to evaluate the relationship between arsenic concentrations in water and toenail arsenic concentration. Demographic variables were also evaluated as predictors of SumAs, AsB, and toenail arsenic concentration.

Separate models were run in the exposure metric analyses to assess different groups. To assess different exposure levels, we grouped individuals according to arsenic concentrations in water: total population (n = 343) and individuals with greater than 1 μg arsenic per l water (n = 137). This grouping was also performed for comparison with previous studies (Karagas et al., 2002; Slotnick et al., 2007). Individuals were further stratified in terms of water intake (plain water plus beverages made with tap water from home); intake was categorized as either greater than or less than the median water intake (2.4 l, n = 80). All statistical analyses were run using the SAS statistical software, version 9.2 (SAS Institute, Cary, NC, USA).

Results

More than half of the participants were men (67.6%) and the average age was 65.7 years (Table 1). As the present analysis took place within a case–control study of bladder cancer, the age and race distribution reflects the fact that bladder cancer is predominantly a disease of elderly white men.

The average storage time for urine samples was 73 days (range: 0–278 days). Storage time was not a predictor of urinary arsenic. Neither MMA[III] nor DMA[III] was detected in any of the samples. Table 1 also shows urinary arsenic (SumAs) as well as toenail and water arsenic concentrations. There were no significant differences in urinary arsenic, drinking water arsenic concentration, and arsenic concentration in toenails between cases and controls, females and males, smokers or non-smokers, or among age categories. Those individuals drinking water from private well excreted 1.6 μg/l (geometric mean) more arsenic than those drinking water from public supplies, a statistically significant difference. Table 2 shows the urinary arsenic metabolite distribution for the study population. SumAs ranged from 0.22 to 74.2 μg/l for urine samples. Inorganic arsenic species (As [III] and As [V]) were the least detected species, whereas AsB was the most abundant species.

Predicting Urinary Arsenic (SumAs)

Population characteristics including age, gender, BMI, and smoking status, were explored as predictors of SumAs. None of these were significant predictors of SumAs. Spearman's correlation procedures show a positive, significant association between SumAs and arsenic concentrations in water (r_S = 0.41, P<0.0001). The correlation increased (r_S = 0.48, P<0.0001) when analyses were run including only individuals with drinking water concentrations exceeding 1 μg/l, and when including only individuals with drinking water concentrations exceeding 1 μg/l and water intake higher than the median (r_S = 0.60, P<0.0001). Single and multiple linear regression models were applied to evaluate predictors of urinary arsenic. All exposure metrics were significant predictors of arsenic concentrations in urine (Table 3). The percentage of variation in SumAs explained by the metrics was similar across all exposure metrics when all participants were included. There was a

substantial increase, however, in the R^2 value of Metric 1 (arsenic in drinking water) for participants with drinking water concentrations ≥1 μg/l and above the median water intake (≥2.4 l per day); Metrics 2 and 3, which included a continuous estimate of water intake within the metrics themselves (e.g., water intake (l per day) × arsenic concentration (μg/l)), did not correlate as well with SumAs. Individual metabolites present the same trend, where Metric 1 increased for participants with drinking water concentrations ≥1 μg/l and above the median water intake (≥2.4 l per day). However, R^2 values for DMA were lower than that for MMA; Metric 1 values for participants with drinking water concentrations ≥1 μg/l and above the median water intake were $R^2 = 0.44$ for MMA and $R^2 = 0.28$ for DMA (results not shown).

A separate multiple regression analysis was performed to explore food consumption and arsenic in urine using estimates of dietary arsenic intake generated from frequency and serving size from the FIQ and arsenic concentrations in food concentration reported in the literature (Supplementary Table S2). Intake of rice, chicken, and canned fish were positively associated with SumAs, but explained only a small percent of the variability (Table 4). It is worth mentioning that chicken intake was borderline associated with SumAs and DMA, but not with AsB. Intake of fish and any seafood were statistically associated with SumAs and therefore individual analyses were directed to evaluate specific arsenic species, as SumAs did not include AsB.

Investigating Urinary AsB, DMA[V], and Food Intake

In addition to its correlation with seafood intake, DMA and AsB showed a positive, significant correlation ($r_S = 0.41$, $P<0.0001$), whereas AsB was not correlated with MMA ($r_S = -0.006$, $P = 0.9138$). Like DMA, AsB was associated with fish and shellfish consumption. Among those who consumed some type of shellfish during the 3 days ($n = 21$), a weak but statistically significant correlation was detected between shellfish consumption and AsB urine concentration ($r_S = 0.15$, $P = 0.0055$). These individuals also had a significantly higher AsB average concentration (21.6 μg/l) compared with the rest of the population (13.3 μg/l). Individuals who consumed any type of fish during the 3 days ($n = 26$) also had a significant correlation between fish consumption and AsB excretion ($r_S = 0.27$, $P<0.0001$). In addition to shellfish and fish, rice was weakly but significantly correlated with urinary AsB concentrations ($r_S = 0.11$, $P = 0.0437$). To assess possible confounding from individuals consuming seafood, we restricted this analysis (rice) to individuals reporting no consumption of seafood and the correlation between rice consumption and AsB persisted ($r_S = 0.11$, $P = 0.0867$). None of the other single food items were significantly correlated with AsB.

Comparing Toenail and Urinary Arsenic Biomarkers

Total arsenic concentration in toenails ranged from below detection limit to 2.1 μg/g. Spearman correlation procedures were applied using arsenic in drinking water and individual urinary arsenic metabolites (Table 5). Water arsenic concentrations showed positive, significant correlations with each arsenic metabolite (except for AsB), with MMA having the highest correlation ($r_S = 0.46$). Similarly, toenail arsenic concentrations showed significant relationships with InAs, MMA[V], and DMA[V], as well as SumAs (Table 5).

Arsenic concentration in water was also correlated with arsenic concentration in toenails (r_S = 0.54, P<0.0001).

Discussion

Consistent with previous findings, arsenic concentration in drinking water was found to be an important factor in predicting urinary inorganic and methylated arsenic. In addition, we found that incorporating home water consumption using categorical cutoffs at the median improved the ability of water arsenic levels to predict urinary concentrations of SumAs. We also found a relationship between DMA[V] and seafood intake, suggesting that future research is needed to better understand the intricacies of arsenic metabolism and dietary sources of arsenic.

Comparing different exposure metrics indicates that arsenic in drinking water is an important predictor of urinary SumAs. The ability of Metric 1 to explain urinary SumAs concentrations increases when drinking water intake is treated categorically (water intake ≥2.4 l in 3 days). When treating drinking water intake as a continuous variable (within Metrics 2 and 3 as l per day × μg/l), the exposure metric did not improve the ability to predict urinary arsenic. A previous attempt to predict urinary arsenic output in a population exposed to slightly higher levels of arsenic in drinking water (arsenic in water ranged from 5.5 to 43.3 μg/l), an increase in water consumption, treated as a continuous variable, resulted in an increase in SumAs (Meza et al., 2004). It is unclear why our study population did not show improved correlation when treating drinking water as a continuous variable. Errors estimating volume of water intake over a 3-day period may lead to possible misclassification, which may be lessened when treating the water intake variable dichotomously into high/low categories.

Independent of water volume, the urinary arsenic metabolites, As[III], As[V], MMA[V], DMA[V], and SumAs, showed positive, significant correlations with a arsenic concentration in drinking water. Similar correlations have previously been reported for SumAs in a population with a comparable concentration of arsenic in water in the United States (Karagas et al., 2001). As all of these metabolites are similarly correlated, it is difficult to determine which metabolite is most strongly related to arsenic in drinking water. It is worth mentioning, however, that MMA has the highest correlation coefficient among the metabolites. MMA excretion has been associated with an increased risk of arsenic-related diseases such as bladder and skin cancer (Hopenhayn-Rich et al., 1996; Chen et al., 2003; Steinmaus et al., 2006). This information supports the hypothesis that arsenic drinking water concentrations are, to some extent, good surrogates of arsenic exposure, even in populations exposed to relatively low levels of arsenic in drinking water (<50 μg/l).

Similar to urinary arsenic, toenail arsenic concentration has been used to determine arsenic exposure (Karagas et al., 2001; Slotnick et al., 2007). Although individuals included in this subsample were different than individuals included in previous subsamples from the main case–control study (Slotnick et al., 2007), correlation coefficients between arsenic in water and arsenic in toenails were very similar (0.56 *versus* 0.54). In addition to the correlation of water and urinary arsenic, toenail arsenic concentration was correlated with SumAs.

This is consistent with previous studies reporting that urinary arsenic concentrations remain constant over long periods of time in populations, with no changes in their drinking water supplies or activity patterns (Navas-Acien et al., 2009).

Arsenic exposure metrics did not include AsB because we mainly wanted to assess inorganic arsenic and because AsB is a less toxic metabolite. AsB does not seem to be metabolized by the human body, but is excreted in urine and is rapidly eliminated unchanged from its consumed form (Le et al., 1993). AsB was not significantly correlated with arsenic concentrations in water ($r_s = -0.15$, $P = 0.1107$) (Table 4). Furthermore, when AsB was included in the sum of TotAs, the correlation between urinary arsenic and arsenic concentration in water was reduced from $r_s = 0.42$ ($P<0.0001$) to $r_s = 0.12$ ($P = 0.0024$). Nonetheless, the identification of organoarsenicals such as AsB assists in the study of dietary sources and arsenic metabolism. In addition, it is worthwhile to consider the potential role of AsB because it may influence other arsenic metabolites such as DMA (Francesconi et al., 2002). Recent studies have reported higher concentrations of DMA in individuals that have consumed fish or seafood during the days before urine sample collection (Choi et al., 2010; Fillol et al., 2010; Navas-Acien et al., 2011).

Our results indicate correlations between seafood intake, AsB, and DMA. As others have suggested (Francesconi et al., 2002; Navas-Acien et al., 2011), these results may indicate that AsB influences the concentration of DMA in urine. In addition, seafood may contain DMA (Devesa et al., 2005), which may be more important for interpreting urinary concentrations in populations exposed to low-to-moderate levels of arsenic in drinking water (<50 μg/l). Understanding the source of DMA in urine of different populations is important because urinary DMA percentage has been used as an indicator of methylation efficiency (Vahter, 1999) and is a carcinogen of the rat bladder (Ma and Le, 1998). If ingestion of organoarsenicals produces or influences DMA, they may need to be considered in toxicological and epidemiological studies. A better understanding of the relationship between seafood intake, AsB, and DMA will also contribute to the recent debate over the merits of whether or not to statistically adjust for urinary AsB in studies of risk from arsenic exposure (Navas-Acien et al., 2008; Steinmaus et al., 2009).

This population does not show, based on the FIQ, high consumption of fish (8%) or shellfish (6%). The geometric means for DMA and AsB (3.8 and 4.4 μg/l, respectively) in our study are very similar to what has been found in the 2000–2003 NHANES (3.8 and 1.8 μg/l, respectively) (Caldwell et al., 2009) and in individuals who did not eat seafood in the 2003–2006 NHANES (3.5 and 1.3 μg/l, respectively) (Navas-Acien et al., 2011). Several studies have also reported elevated concentrations of urinary AsB in populations that consumed little or no fish or seafood (Lai et al., 2004; Brima et al., 2006). Our results may also point to additional non-seafood sources of AsB. Lai et al. (2004) explain that slow excretion of AsB or the ability to metabolize arsenic compounds through different pathways may be the reason for high amounts of AsB in volunteers who refrained from eating fish or seafood 3 days before the urine collection. It is also possible that there is consumption of hidden fish products (Ritsema et al., 1998), such as some barbeque sauces and multivitamins, unknown to study participants. This suggests that more work is needed to better understand

dietary sources of arsenic species to appropriately use these urinary species as biomarkers of exposure in studies of arsenic-related risk.

There were some limitations that can be identified in this study. Recall error may have been introduced through the FIQ when information on diet and water intake was collected. Measurement error may also be present owing to arsenic in food data obtained from the literature. Data on inorganic arsenic in consumed foods in the United States of America are limited and vary by region (Schoof et al., 1999). The lack of available data may affect the results related to inorganic arsenic and food consumption. In addition, other food sources of arsenic may not have been captured by the FIQ. In spite of these limitations, this study reveals positive, significant correlation between urinary arsenic and drinking water arsenic concentrations in a population exposed to low levels of arsenic in their drinking water. The use of the FIQ, where exposure through other pathways was examined, adds information about exposure besides arsenic in their drinking water alone. In addition, this population was not a high consumer of fish or seafood and still had detectable levels of AsB. The correlations between seafood intake, AsB, and DMA deserve further study, even in populations with relatively low consumption of seafood. The marginal correlation between chicken intake, SumAs, and DMA deserves further study as well given the debate regarding the potential contribution of arsenicals used in animal feed to total human exposure (Silbergeld and Nachman, 2008). Additional studies with more statistical power are needed to evaluate this finding. This research may lead to improved selection of appropriate biomarkers in future research, accounting for logistical issues, budgetary concerns, and laboratory facilities. These results advance the validation process of urinary arsenic species as biomarkers of arsenic exposure, which is critical before the widespread application of this tool in risk assessment and epidemiological studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Geometric means of arsenic in urine (SumAs), arsenic in water, and arsenic in toenails for the study population.

Population characteristics	*N*	%	[As] water (μg/l)	Urinary SumAs[a] (μg/l)	[As] toenails (μg/g)
Total population	343	100	0.74	5.02	0.09
Disease (bladder cancer) status					
Cases	151	44.0	0.83	4.72	0.09
Controls	192	66.0	0.66	5.26	0.09
Age (years)					
<55	49	14.3	1.24	5.03	0.11
55–64	84	24.4	1.09	5.41	0.12
65–74	114	33.3	0.44	4.68	0.08
≥75	96	28	0.76	5.10	0.07
Gender					
Male	232	67.6	0.81	5.15	0.10
Female	111	32.3	0.59	4.75	0.08
Race					
White	324	95.3	0.70	5.00	0.09
Other	15	4.7	1.15	7.00	0.14
Current smoker					
Yes	211	10.7	0.81	5.11	0.10
No	132	89.2	0.62	4.92	0.08
BMI					
<25	140	40.8	0.84	4.78	0.09
25–30	147	42.8	0.62	5.10	0.10
≥30	56	16.3	0.82	5.42	0.08
Drinking water source					
Public supply surface	102	35.3	0.41	4.22	0.05
Private well	202	58.9	1.39	5.79[b]	0.13
Public supply well	5	1.2	0.09	4.18	0.06
Bottle	33	9.6	0.09	4.18	0.06
Missing	1	0.0	0.49	2.30	0.07

[a] SumAs: sum of As[III], As[V], MMA[V] and DMA[V].

[b] Statistically significant at $\alpha = 0.05$.

Table 2.

Arsenic species in urine (μg/l) from 343 participants from southeastern Michigan.

Arsenic	G mean	A[a] mean	Range	25th	75th	95th
Urinary As[III]	0.14	0.29	<0.11–3.40	<0.11	0.35	1.1
Urinary As[V]	0.12	0.19	<0.15–2.14	<0.15	<0.15	0.82
Urinary MMA[V]	0.56	1.09	<0.12–18.0	0.35	1.21	3.7
Urinary DMA[V]	3.81	5.53	<0.08–74.0	2.22	6.85	15.4
Urinary AsB	4.43	13.3	<0.06–257.0	1.79	10.32	47.1
TotAs	11.54	20.4	<0.07–331.2	5.83	21.40	61.7
SumAs	5.01	7.10	0.22–74.2	3.00	8.48	20.4

Abbreviations: G mean, geometric mean; A mean, arithmetic mean; TotAs, sum of As[III], As[V], MMA[V], DMA[V] and AsB; SumAs, sum of As[III], As[V], MMA[V] and DMA[V].

[a]Detection limits for arsenic species: As[III], 0.11 μg/l; As[V], 0.15 μg/l; MMA[V], 0.12 μg/l; DMA[V], 0.08 μg/l; AsB, 0.06 μg/l; and TotAs, 0.07 μg/l.

Table 3.

Relationship between urinary arsenic concentrations (SumAs, μg/l) and estimated arsenic exposure, stratified by arsenic drinking water concentration and water intake.

Drinking water arsenic concentration and intake	*N*	Metric	*β* Estimate	Model results[a,b], R^2
All samples	339	(1) AsDW	0.2523	0.1827
	339	(2) AsDW × DWI	0.0162	0.1959
	339	(3) AsDW × DWI+BI	0.0097	0.1964
	339	(4) AsDW × DWI+FoodAs	0.0098	0.1953
≥1 μg/l[c]	137	(1) AsDW	0.3830	0.2902
	137	(2) AsDW × DWI	0.0144	0.2733
	137	(3) AsDW × DWI+BI	0.0087	0.2713
	137	(4) AsDW × DWI+FoodAs	0.0087	0.2717
≥1 μg/l and total water intake ≥Median (2.4 lin 3 days)[d]	80	(1) AsDW	0.4982	0.4025
	80	(2) AsDW × DWI	0.0141	0.3092
	80	(3) AsDW × DWI+BI	0.0086	0.2954
	80	(4) AsDW × DWI+FoodAs	0.0087	0.2958

Abbreviations: AsDW, arsenic concentration in home drinking water sample (μg/l); BI, intake of beverages other than plain water made from tap water at home (l per day); DWI, intake of plain drinking water at home (l per day); FoodAs, estimated concentration of inorganic arsenic in food (μg per day).

[a] Adjusted by water source.

[b] All statistically significant at $\alpha = 0.05$.

[c] Participants with more than 1 μg/l in their drinking water.

[d] Total water intake = plain water+beverages made with tap water.

Table 4.

Estimates of arsenic in food[a] as predictors of SumAs (μg/l), AsB (μg/l), and DMA[V] (μg/l).

Variable	N	SumAs		AsB		DMA[V]	
		β Estimate	Model R^2 (*P*-value)	β Estimate	Model R^2 (*P*-value)	β Estimate	Model R^2 (*P*-value)
All Individuals							
Total Food	343	1.1	0.068 (<0.0001)	0.65	0.025 (0.015)	0.94	0.078 (0.033)
Individual food items							
Rice	35	7.51	0.008 (0.098)	15.11	0.012 (0.044)	8.33	0.010 (0.067)
Chicken	96	0.63	0.009 (0.086)	0.39	0.001 (0.498)	0.63	0.009 (0.087)
Mushrooms	99	−14.02	0.002 (0.412)	25.44	0.002 (0.367)	−13.28	0.002 (0.431)
Canned Fish	11	53.39	0.005 (0.193)	205.52	0.027 (0.002)	38.51	0.020 (0.125)
Shellfish	21	20.08	0.010 (0.070)	55.18	0.032 (0.009)	23.12	0.014 (0.052)
Fish	26	0.71	0.015 (0.025)	2.94	0.103 (<0.0001)	0.65	0.021 (0.036)
Any Seafood	55	0.71	0.015 (0.022)	2.58	0.073 (<0.0001)	0.52	0.016 (0.014)

[a]We assessed food intake by multiplying the frequency of food consumption and the typical portion size by the arsenic content reported in the literature (μg per day).

Table 5.

Spearman's correlations of urinary arsenic metabolites with arsenic in water and toenail (n = 343).

Marker	Arsenic metabolites in urine					
	As[III]+As[V] (μg/l)	MMA[V] (μg/l)	DMA[V] (μg/l)	AsB (μg/l)	TotAs (μg/l)	SumAs (μg/l)
Water	0.370	0.463	0.387	–0.147*	0.122	0.416
Toenails	0.351	0.369	0.312	–0.092*	0.125	0.345

*P-value >0.05.

All other correlations P<0.05.