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Seafood Intake and Urine Concentrations of Total Arsenic, Dimethylarsinate and Arsenobetaine in the US Population

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Abstract

Background—Seafood is the main source of organic arsenic exposure (arsenobetaine, arsenosugars and arsenolipids) in the population. Arsenosugars and arsenolipids are metabolized to several species including dimethylarsinate (DMA).

Objective—Evaluate the association of seafood intake with spot urine arsenic concentrations in the 2003–2006 National Health Nutrition and Examination Survey (NHANES).

Methods—We studied 4276 participants ≥ 6 y. Total arsenic was measured using inductively coupled plasma dynamic reaction cell mass spectrometry (ICPMS). Urine DMA and arsenobetaine were measured by high-performance liquid chromatography coupled with ICPMS.

Results—Participants reporting seafood in the past 24-h had higher urine concentrations of total arsenic (median 24.5 vs. 7.3 $\mu\text{g/L}$), DMA (6.0 vs. 3.5 $\mu\text{g/L}$), arsenobetaine (10.2 vs. 0.9 $\mu\text{g/L}$) and total arsenic minus arsenobetaine (11.0 vs. 5.5 $\mu\text{g/L}$). Participants reporting seafood $\geq 2/\text{wk}$ vs. never during the past year had 2.3 (95% confidence interval 1.9, 2.7), 1.4 (1.2, 1.6), 6.0 (4.6, 7.8) and 1.7 (1.4, 2.0) times higher (p-trend < 0.001) concentrations of total arsenic, DMA, arsenobetaine and total arsenic minus arsenobetaine, respectively. In participants without detectable arsenobetaine and in analyses adjusted for arsenobetaine, seafood consumption in the past year was not associated with total arsenic or DMA concentrations in urine.

Conclusion—Seafood intake was a major determinant of increased urine concentrations of total arsenic, DMA, arsenobetaine and total arsenic minus arsenobetaine in the US population.

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Epidemiologic studies that use total arsenic, DMA, the sum of inorganic arsenic, methylarsonate and DMA, and total arsenic minus arsenobetaine as markers of inorganic arsenic exposure and/or metabolism need to address seafood intake.

Keywords

arsenic; arsenobetaine; dimethylarsinate; NHANES; seafood

INTRODUCTION

Humans are exposed to different forms of arsenic, including inorganic (arsenite, arsenate) and organic (arsenobetaine, arsenosugars and arsenolipids) compounds. The sources of exposure, biotransformation in the human body and toxicity of inorganic and organic arsenic differ markedly (Cullen and Reimer 1989; Fowler et al. 2007). Inorganic arsenic is an established human carcinogen that is also associated with increased developmental, cardiovascular and metabolic disease (International Agency for Research on Cancer 2002; Navas-Acien et al. 2005; Navas-Acien et al. 2006; Navas-Acien et al. 2009a; States et al. 2008; Wasserman et al. 2004). The main sources of inorganic arsenic for general populations worldwide are drinking water and food (rice and grains) (EFSA CONTAM 2009; IPCS (International Programme on Chemical Safety) 2001; National research council 1999). Arsenite and arsenate are methylated in the human body in two steps, resulting in the formation of methylarsonate and dimethylarsinate (DMA) which are excreted in urine together with unchanged inorganic arsenic (Cullen and Reimer 1989; Vahter 2002).

Seafood is the main source of organic arsenic compounds (Cullen and Reimer 1989; Francesconi and Kuehnelt 2004). Most fish and shellfish have high tissue levels of arsenobetaine, a non-toxic arsenical (Sabbioni et al. 1991) that in humans is excreted unchanged via the kidneys contributing to total urine arsenic. In addition to containing arsenobetaine, seaweed and mollusks are rich in arsenosugars (Francesconi and Edmonds 1996) and fatty fishes are rich in arsenolipids (Schmeisser et al. 2005; Taleshi et al. 2009). In humans, arsenosugars and arsenolipids are metabolized to several arsenic species including oxo-arsenicals, thio-arsenicals and, predominantly, DMA (Raml et al. 2005; Schmeisser et al. 2006). DMA is thus a common metabolite that reflects exposure to both inorganic and organic arsenic compounds (Choi et al. 2010; Ma and Le 1998; Navas-Acien et al. 2009a).

Few population-based studies have evaluated the contribution of seafood intake to total urine arsenic and urine arsenic species concentrations in the general population. This is an important question because urine is commonly used in epidemiologic studies to investigate inorganic arsenic exposure (by measuring total arsenic or the sum of arsenite, arsenate, methylarsonate and DMA species) and metabolism (e.g. by measuring the percentage of methylarsonate or DMA over the sum of inorganic and methylated species). Moreover, total urine arsenic minus arsenobetaine has been used as a marker of inorganic arsenic exposure in several studies (Ahsan et al. 2007; Brima et al. 2006; Gamble et al. 2005; Steinmaus et al. 2009). In populations with seafood intake, however, subtracting arsenobetaine may not be sufficient to remove the contribution of arsenosugars and arsenolipids to DMA and other arsenic metabolites in urine.

In the present study, conducted in a representative sample of the US population, we hypothesized that in addition to increased arsenobetaine and total arsenic concentrations in urine, participants reporting seafood intake in the past 24-h would have increased urine concentrations of DMA and total arsenic minus arsenobetaine, likely as a result of arsenosugar and arsenolipid metabolism (Figure 1). We also evaluated urine concentrations

of total arsenic, DMA, arsenobetaine and total arsenic minus arsenobetaine according to self-reported frequency of seafood consumption during the past year using several strategies to evaluate the contribution of recent seafood intake to total urine arsenic and urine DMA concentrations.

METHODS

Study population

We analyzed data from the National Health Nutrition and Examination Survey (NHANES) 2003–2006 conducted by the US National Center for Health Statistics. The National Center for Health Statistics used a complex multistage sampling design to obtain a representative sample of the civilian non-institutionalized US population (National Center for Health Statistics 2009). The 2003–2006 NHANES study protocols were approved by the National Center for Health Statistics institutional review board. Oral and written informed consent was obtained from all participants. The participation rate in NHANES 2003–2006 interviews and physical examinations was 77%.

Seafood intake in the past 24-h

Seafood intake during the past 24-h was obtained during dietary recall interviews conducted in English or Spanish using an automated multiple pass method (National Center for Health Statistics 2002). Multiple pass 24-h recall is the method of choice to determine recent food intake as well as the average food intake of large population samples (Institute of Medicine 2000). The dietary recall was conducted in person during the examination component of NHANES. The primary goal was to record details of all foods and beverages the participant consumed within a 24-h period, indicating week day and seasonality. Trained interviewers obtained the most complete data directly from the participant, including assistance from an adult (preferably the person responsible for preparing the participant's meals) for all participants 6–11 y and when necessary for participants ≥ 12 y. The 24-hour dietary recall was divided into 5 steps: (1) quick list of foods easily remembered by the participant, (2) frequently forgotten foods list such as beverages, snacks, cheese or bread (seafood was not included in this list), (3) time and occasion questions for additional recalling according to eating patterns, (4) detail and review cycle for each food and eating occasion and (5) final review probe asking once more about anything else they ate or drank. Around 5% of the dietary interviews, randomly selected, were audiotaped and reviewed centrally for quality control. Other quality control measures included field observations, data retrieval and review of data collected. Dietary interviewers entered the information into an automated dietary recall system with a list of > 2,600 foods, including many ethnic foods.

For each food item reported by the participant during the past 24-h, a US Department of Agriculture food code was assigned including information about whether the food was eaten in mixture with other foods (e.g. sandwich, soup) (US Department of Agriculture.Agriculture Research Service 2008). Using the US Department of Agriculture food codes, we identified participants who reported any seafood intake as an individual item or in mixtures (Appendix 1). In addition to overall seafood intake (any seafood), we grouped participants who reported (1) fish, (2) shellfish (including mollusks), (3) mollusks and (4) sushi or seaweed, as individual food items.

Seafood frequency during the past year

An English or Spanish 124-item food frequency questionnaire developed by the National Cancer Institute, National Institutes of Health (Subar et al. 2001) was sent by mail to the participants' homes to ascertain information on food consumption patterns during the past year. A postage-paid envelope was included and compensation was provided for participants

who returned their questionnaires. The questionnaire included only 6 seafood items: tuna, smoked fish, sushi, raw oysters, fried fish, and all other fish including shellfish combined. We grouped the responses to the 6 items in one group of overall seafood frequency consumption during the past year categorized as never, 1–11 times per year (<1 per month), 1–4 times per month, ≥ 2 times per week. Quality control programs and manual verification checks were used to verify the completeness and accuracy of the data before scanning the returned questionnaires.

Urine arsenic

Spot urine samples for arsenic analysis were collected in arsenic-free containers, shipped on dry ice, stored frozen at $\leq -70^{\circ}\text{C}$ and analyzed within 3 weeks of collection (Caldwell et al. 2009). Urine collection and storage materials were screened for arsenic contamination before use (National Center for Health Statistics 2008). Total arsenic and arsenic species were measured at the Environmental Health Sciences Laboratory of the National Center for Environmental Health following a standardized protocol (National Center for Environmental Health 2008a). Their concentrations in urine were reported as arsenic atom equivalent concentrations. Total urine arsenic was measured using inductively coupled-plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS) on a Perkin-Elmer ELAN[®] 6100 DRC^{PLUS} or ELAN[®] DRC II ICPMS (Perkin Elmer SCIEX, Concord, ON, Canada) (Caldwell et al. 2009). The limit of detection was 0.6 $\mu\text{g/L}$ in NHANES 2003–2004 and 0.74 $\mu\text{g/L}$ in NHANES 2005–2006. The percentage of participants with total urine arsenic levels below the limit of detection was 1.0% and 0.7% in NHANES 2002–2004 and NHANES 2005–2006, respectively. National Institute of Standards and Technology (NIST) standard reference material (SRM) 2670 (elevated) was used for external calibration verification. The inter-assay coefficients of variation for quality control pooled samples analyzed throughout the duration of the survey were 9.2% and 19.4% for total urine arsenic lots with mean arsenic levels 8.15 $\mu\text{g/L}$ and 4.07 $\mu\text{g/L}$, respectively (National Center for Environmental Health 2008a).

In NHANES 2003–2006, the following urinary arsenic species were measured by high performance liquid chromatography (HPLC)-ICP-DRC-MS (National Center for Environmental Health 2008b): arsenite (limit of detection=1.2 $\mu\text{g/L}$), arsenate (1.0 $\mu\text{g/L}$), methylarsonate (0.9 $\mu\text{g/L}$), DMA (1.7 $\mu\text{g/L}$), arsenobetaine (0.4 $\mu\text{g/L}$) and arsenocholine (0.6 $\mu\text{g/L}$). The percent study participants with detectable levels of arsenite, arsenate, methylarsonate and arsenocholine were only 4.5%, 5.7%, 37.5% and 2.1%, respectively. As a consequence, these four arsenic species were not used in our analyses. We note, however, that arsenite, arsenate and methylarsonate are not relevant species in seafood and have not been found as metabolites of arsenosugars or arsenolipids in experimental studies (Choi et al. 2010; Heinrich-Ramm et al. 2002; Lai et al. 2004). Although arsenocholine is sometimes reported in seafood, it is usually present at trace levels only (Cullen and Reimer 1989), and it is rarely detected in urine (Navas-Acien 2009a). Most study participants had detectable levels of DMA (87.9%) and arsenobetaine (67.5%), and these species were used in data analysis. Participants with undetectable arsenobetaine were less likely to report seafood intake in the past 24-h (only 1.9%) compared to participants with detectable arsenobetaine (20.1%). The inter-assay coefficients of variation for quality control pooled samples with mean DMA of 6.66 $\mu\text{g/L}$ and mean arsenobetaine of 4.87 $\mu\text{g/L}$ were 7% and 10%, respectively (National Center for Environmental Health 2008b). Participants with total arsenic, DMA or arsenobetaine levels below the limit of detection were assigned values equal to the limit of detection divided by the squared root of two (Hornung and Reed 1990; US Department of Health and Human Services 2005).

Other variables

Questionnaire information included sex, age, race/ethnicity, education (of the participant if ≥ 20 years old or of the household reference person if <20 years old) and smoking status (never / former / current). Participants ≥ 20 years old were classified as never, former and current smokers using the questions “Have you smoked at least 100 cigarettes in your entire life?” and “Do you now smoke cigarettes every day, some days or not at all”. Participants 12–20 years old were classified as current, former and never smokers using the questions “How old were you when you smoked a whole cigarette for the first time” and “During the past 30 days, on how many days did you smoke cigarettes”. Serum cotinine concentrations were measured by an isotope-dilution HPLC/atmospheric pressure chemical ionization tandem mass spectrometric method. A level equal to the limit of detection divided by the squared root of two was assigned to participants with levels below the limit of detection for serum cotinine (18.3% below $0.015 \mu\text{g/L}$). All participants with serum cotinine $>10 \mu\text{g/L}$ were reclassified as current smokers independent of their self-reported smoking status or age (Benowitz 1996).

Body mass index was calculated by dividing measured weight in kilograms by measured height in meters squared and standardized by age and sex for participants ≤ 18 years old. Body mass index (BMI) was categorized as normal ($<25 \text{ kg/m}^2$), overweight (25 to $<30 \text{ kg/m}^2$) and obese ($\geq 30 \text{ kg/m}^2$) for participants 19 years of age and older, and as normal ($\text{BMI} < 85^{\text{th}}$ percentile), overweight ($\text{BMI} \geq 85^{\text{th}}$ percentile and $< 95^{\text{th}}$ percentile) and obese (body mass index $\geq 95^{\text{th}}$ percentile) for participants 6 to 18 years old based on the CDC’s BMI-for-age and sex specific growth charts (Centers for Disease Control and Prevention 2010).

Blood mercury was used as an objective marker of seafood intake (Mahaffey et al. 2008). Total blood mercury concentrations were measured by quadrupole ICPMS (National Center for Environmental Health 2004). A level equal to the limit of detection divided by the squared root of two was assigned to participants with levels below the limit of detection for blood mercury (8.0% below $0.2 \mu\text{g/L}$) (Hornung and Reed 1990; US Department of Health and Human Services 2005).

Urine creatinine, used to account for urine dilution in spot urine samples, was determined using a Jaffé rate reaction measured with a Beckman CX3 analyzer.

Statistical analysis

All statistical analyses were performed using the survey package in R to account for the complex sampling design (Lumley 2008). Strata, primary sampling units, and special sample weights for arsenic analyses were used to obtain unbiased point estimates and robust linearized standard errors.

Total arsenic, DMA, arsenobetaine and total arsenic minus arsenobetaine concentrations were right skewed and log-transformed for the statistical analyses. For each arsenical, we estimated the ratio of the geometric mean arsenic concentrations and its 95% confidence interval (CI) comparing seafood intake (any seafood and specific items) to no seafood intake in the past 24-h using linear regression models on log-transformed arsenic levels. The ratios of the geometric means (95% confidence intervals) were obtained by exponentiating the coefficients and standard errors from the linear regression models on log-transformed arsenic. The models were performed with the following levels of adjustment: crude, adjusted for creatinine (continuous), and further adjusted for age (continuous), sex (men / women), race / ethnicity (white / black / Mexican-American / other), education ($<$ high school / high school or equivalent / $>$ high school), body mass index (continuous), smoking status (never / former / current) and serum cotinine (log-transformed). Crude and adjusted models showed

similar results and we selected to present crude geometric means (95% CI) and fully adjusted ratios (95% CI) of geometric means. Using similar modeling, we also estimated the ratio (95% CI) of the geometric mean arsenic concentrations comparing participants with increasing categories of seafood intake frequency (1–11 times per year, 1–4 times per month, ≥ 2 times per week) to no seafood in the past year. A test for trend in the association between increasing seafood frequency and arsenic concentrations was conducted by adding the frequency of seafood intake as a continuous variable to the model.

The frequency of seafood intake over the past year could be related to urine arsenic biomarkers because frequent seafood intake over the past year can be associated with recent seafood intake (Figure 1). To evaluate methods for removing the contribution of recent seafood intake to urine arsenic biomarkers we used three strategies. First, we restricted the analysis of the association of frequency of seafood intake in the past year with urine arsenic to participants who self-reported no seafood intake in the past 24-h. Second, we restricted the analysis to participants with undetectable arsenobetaine (half-life of arsenobetaine is around 1–2 days for most individuals). Third, we conducted the analysis in the overall sample but adding further adjustment for urine arsenobetaine as a marker of recent seafood intake. In this third model, total arsenic and DMA now reflect arsenic that is not related to seafood.

Stratified analyses by age group (<20, 20–44, 45–64, ≥ 65 y), sex (men / women), race/ethnicity (white / black / Mexican-American / other), education (<high school / high school and equivalent / >high school), body mass index (normal / overweight / obese), and smoking status (never / former / current) yielded similar results (data not shown). Finally, we evaluated the potential impact of participants below the limit of detection for DMA and arsenobetaine restricting the analyses to participants above the limit of detection for DMA and arsenobetaine with similar results (data not shown).

RESULTS

Study population

NHANES 2003–2006 randomly selected a one-third random sample of study participants aged 6 years and older for arsenic measures (N=5365). We selected 5048 participants who had participated in a dietary recall interview for the past 24 hours prior to urine collection. We then excluded 188 pregnant women, 168 participants missing values for total urine arsenic or urine arsenic species, and 416 participants missing other variables of interest (education, body mass index, serum cotinine, smoking status and blood mercury), leaving a total of 4276 participants for analysis based on dietary recall. For analysis based on the food frequency questionnaire, we further excluded 1018 participants missing data on seafood consumption over the past year, leaving 3258 participants for food frequency questionnaire analysis. Study participants for 24-h dietary recall and food frequency questionnaire analyses were similar by age, sex, race, education and body mass index with respect to NHANES 2003–2006 participants selected for arsenic analyses (data not shown).

Seafood intake in the past 24-h

A total of 14.6% of the population reported any seafood intake in the past 24-h (Table 1). Participants reporting any seafood intake in the past 24-h were older, more likely female, less likely white and Mexican-American, more educated, more likely to eat seafood >1/ month and had lower serum cotinine concentrations and higher blood mercury concentrations compared to participants who reported no seafood intake in the past 24-h. Participants reporting any seafood intake in the past 24-h also had increased levels of all urine arsenic biomarkers included in the study compared to participants with no seafood

intake: total arsenic (median 24.5 vs. 7.3 µg/L), DMA (6.0 vs. 3.5 µg/L), arsenobetaine (10.2 vs. 0.9 µg/L) and total arsenic minus arsenobetaine (11.0 vs. 5.5 µg/L). Arsenobetaine was detected in 96.5% participants who reported seafood intake in the past 24-h and in 64.9% of those who did not. Urine arsenobetaine concentrations were strongly related to total urine arsenic concentrations (spearman correlation coefficient =0.78, p-value<0.001), and moderately related to urine concentrations of DMA (r=0.46, p-value 0.001) and total arsenic minus arsenobetaine (r=0.49, p-value <0.001), after controlling for urine dilution (Figure 2). Blood mercury levels were also moderately correlated with urine concentrations of total arsenic (r=0.43, p-value <0.001), DMA (r=0.27, p-value <0.001) and total arsenic minus arsenobetaine (r=0.31, p-value<0.001).

When different types of seafood were analyzed separately (any seafood, fish, shellfish, mollusks, sushi/seaweed), urine concentrations of total arsenic, DMA, arsenobetaine and total arsenic minus arsenobetaine were markedly increased for all types of seafood (Table 2, Figure 3). Geometric mean of total urine arsenic concentrations were 3.5, 3.8, 4.9, 7.0 and 5.5 times higher comparing any seafood, fish, shellfish, mollusks, and sushi/seaweed, respectively, to no seafood in the past 24-h after adjustment for urine creatinine, sociodemographic and smoking status (Table 2). The corresponding ratios were 1.9, 1.9, 2.3, 3.8 and 3.2, respectively, for DMA; 7.7, 7.7, 12.1, 15.8 and 9.2, respectively, for arsenobetaine; and 2.3, 2.5, 3.0, 5.2 and 5.2, respectively, for total arsenic minus arsenobetaine.

Frequency of seafood intake in the past year

70.0% of the population reported seafood intake at least once a month during the past year (Table 1). Compared to participants reporting seafood intake <1/month, participants who reported seafood intake ≥ 1/month had higher median concentrations for all measured urine arsenic biomarkers: total arsenic (9.0 vs. 6.6 µg/L), DMA (4.0 vs. 3.1 µg/L), arsenobetaine (1.9 vs. 0.5 µg/L), and total arsenic minus arsenobetaine (6.0 vs. 5.2 µg/L). Urine concentrations of total arsenic, DMA, arsenobetaine and total arsenic minus arsenobetaine increased with increasing frequency of reported seafood consumption in the past year (Table 3). The percentage of participants with detectable arsenobetaine was 25.4% among those reporting no seafood during the past year and 59.4%, 75.0% and 84.8%, respectively, among those reporting seafood less than once a month, 1–4 times per month and at least twice a week in the past year.

When we restricted the analysis to participants reporting no seafood intake in the past 24-h (N=2821), a positive dose-response relationship between frequency of seafood intake and urine arsenic measurements was still evident (Table 4). However, when we restricted the analysis to participants with undetectable arsenobetaine (N=1391), the frequency of seafood consumption in the past year was no longer associated with total urine arsenic or urine DMA (Table 4). Consistent with the restriction strategy, increasing frequency of seafood consumption in the past year was no longer associated with urine concentrations of total arsenic, DMA or total arsenic minus arsenobetaine in analyses conducted in the overall study sample (N=3258) after adjustment for urine arsenobetaine concentrations.

DISCUSSION

Recent seafood consumption resulted in markedly increased urine concentrations of total arsenic, DMA, arsenobetaine, and total arsenic minus arsenobetaine in a representative sample of the US population ≥ 6 years of age. These findings indicate that recent seafood intake contributes to increased urine DMA concentrations in the general population. This is consistent with human experimental studies reporting increased urine concentrations of DMA and other arsenic species after seafood intake (Choi et al. 2010; Heinrich-Ramm et al.

2002; Lai et al. 2004; Le et al. 1994; Ma and Le 1998), most likely due to human metabolism of arsenosugars and arsenolipids (Francesconi et al. 2002; Raml et al. 2005; Raml et al. 2009; Schmeisser et al. 2006). These findings also indicate that total urine arsenic minus arsenobetaine is not an appropriate biomarker of inorganic arsenic exposure in the US population, since this index remained strongly associated with seafood intake. Among participants with undetectable arsenobetaine, on the other hand, the frequency of seafood intake in the past year was no longer associated with total arsenic or DMA concentrations in urine, supporting that this strategy can effectively eliminate the contribution of organic arsenicals of marine origin to total urine arsenic and urine DMA concentrations. The results among participants with undetectable arsenobetaine were similar to those in the overall sample after adjustment for arsenobetaine and are likely explained by the fact that seafood is a common source of arsenobetaine, arsenosugars and arsenolipids. Among participants with undetectable arsenobetaine, total urine arsenic and urine DMA concentrations likely reflect exposure to arsenic that is not derived from seafood.

DMA is the most abundant arsenic species occurring in urine after exposure to inorganic arsenic as a result of arsenic metabolism (Vahter 2002). In the absence of seafood intake, multiple studies have shown that ~60–80% of total arsenic in urine is DMA (Chiou et al. 1997; Del Razo et al. 1997; Hopenhayn-Rich et al. 1996; Navas-Acien et al. 2009b; Vahter 2002). Urine DMA, however, also reflects seafood intake. Human experiments exposing volunteers to controlled amounts of seaweed (Ma and Le 1998), mussels (Lai et al. 2004), fish including herring (Heinrich-Ramm et al. 2002), seafood including seaweed (Choi et al. 2010; Le et al. 1994), and cod liver (Schmeisser et al. 2006) have shown marked increases in urine DMA concentrations for several days following ingestion. The DMA content in human urine was much larger than DMA measured in seafood before ingestion (Heinrich-Ramm et al. 2002; Lai et al. 2004; Le et al. 1994; Schmeisser et al. 2006), supporting the hypothesis that urine DMA was a result of arsenosugar and arsenolipid metabolism in the human body. Indeed, human experiments exposing volunteers to controlled amounts of synthesized arsenosugars (Francesconi et al. 2002; Raml et al. 2005; Raml et al. 2009) and arsenolipids from cod liver (Schmeisser et al. 2006) have confirmed that DMA is a major metabolite of arsenosugars and arsenolipids. DMA in urine can thus reflect both exposure to inorganic (arsenite, arsenate) and organic (arsenosugars, arsenolipids) arsenic compounds. Direct exposure to DMA, on the other hand, is nowadays uncommon because water, seafood (Sirot et al. 2009) and terrestrial foods (crops, fruit, meat) are generally low in DMA, although some cultivars of rice contain DMA together with inorganic arsenic (Williams et al. 2005).

The methylation of inorganic arsenic to DMA was traditionally considered a detoxification pathway because DMA is more rapidly eliminated through urine and is of much lower toxicity in experimental models compared to inorganic and trivalent methylated arsenic species (Styblo et al. 2000; Vahter 2000). In studies of inorganic arsenic metabolism and health endpoints, individuals with higher proportions of DMA in urine compared to other species have lower risk of skin lesions, skin cancer, bladder cancer, peripheral arterial disease and atherosclerosis (Ahsan et al. 2007; Huang et al. 2009; McCarty et al. 2007; Steinmaus et al. 2006; Tseng et al. 2005; Yu et al. 2000). While many of these studies have been conducted in populations with low seafood intake, the possibility that DMA in urine was the result of organic arsenic (arsenosugars, arsenolipids) metabolism was not evaluated. In a study in Taiwan, the %DMA in urine was similar before and after refraining from seafood intake for three days, however, the relationship between the frequency of seafood and seaweed intake with urine DMA concentrations was not reported (Hsueh et al. 2002).

In our study, participants who reported any type of fish or shellfish (crustaceans and mollusks) in the previous 24-h had urine DMA concentrations 1.8 times higher compared to those reporting no seafood intake in the past 24-h. For those who specifically reported

having mollusks and sushi/seaweed, urine DMA concentrations were more than 3 times higher. Around 75 percent participants who reported mollusk and ~50 percent of those who reported sushi/seaweed had DMA higher than 10 µg/L. These findings are consistent with a study among 101 male office workers in Hamburg, Germany (Heinrich-Ramm et al. 2001) in which urine DMA concentrations were 3 times higher for participants who ate seafood (mostly herring) in the previous 6 days (mean 14.7 µg/L) compared with those who had not eaten seafood in the previous 6 days (mean 4.6 µg/L).

A concern in the risk assessment of DMA, whether it arises from inorganic arsenic in drinking water or organic arsenic in seafood, is its potential to be reduced to the more reactive and toxic DMA(III) species (Valenzuela et al. 2005). Other concerns related to arsenosugar and arsenolipid exposures include their complex metabolism, their large inter-individual variability in half-life and resulting urine species, and their incomplete toxicological assessment (Choi et al. 2010; Raml et al. 2009). In our study, other arsenic species related to arsenosugar and arsenolipid metabolism (e.g. oxo-arsenicals, thio-arsenicals) were likely contributing to total urine arsenic minus arsenobetaine.

Few studies have measured urine arsenobetaine concentrations in general populations, despite important advances to quantify arsenobetaine in biological samples. In Italy, median urine arsenobetaine concentrations ranged between 9.5 and 36.0 µg/L in men from 2 general populations with different levels of seafood intake (Apostoli et al. 1999; Soleo et al. 2008). In a healthy volunteer study in Leicester, UK that requested no seafood intake for 3 days, median arsenobetaine concentrations were 7.8 µg/g creatinine in participants of Asian descent, 2.4 µg/g creatinine in participants of European descent and 0.4 µg/g creatinine in participants of African descent (Brima et al. 2006). Seafood frequency, however, was not used to assess differences in arsenobetaine and in total arsenic minus arsenobetaine between the different racial/ethnic groups (Brima et al. 2006). In our study, urine arsenobetaine concentrations increased steadily with self-reported frequency of seafood consumption in the past year even after restricting the analysis to participants with no self-reported seafood intake in the past 24-h. In participants who reported no seafood intake in the past year, arsenobetaine concentrations were very low but still detectable in 25.4% of the study population. The presence of arsenobetaine in the urine of those participants could be related to incomplete participant recall, to incomplete seafood assessment in the food frequency questionnaire, or to potential presence of arsenobetaine in sources that are unknown or difficult to collect with a food frequency questionnaire. Also, while arsenobetaine is found almost exclusively in marine organisms, it has also been measured in some types of mushrooms (Byrne et al. 1995; Smith et al. 2007).

Strengths of our study include the large sample size, the representative sample of the US population including children and adults, the assessment of past-24 h and past-year seafood intake, and the analytical methods used for measuring total arsenic, DMA and arsenobetaine. There were, however, some limitations. First, we could not evaluate the association of seafood intake with arsenite, arsenate and methylarsonate concentrations in urine since most of the measurements were below the limit of detection. Previous human experiments, however, do not suggest changes in urine concentrations of inorganic arsenic species or methylarsonate after seafood intake (Choi et al. 2010; Heinrich-Ramm et al. 2002; Lai et al. 2004). A second limitation was the use of a spot urine sample, although the results were similar with or without adding urine creatinine to control for urine dilution. Third, water and other dietary sources of arsenic (e.g. rice and grains) were not evaluated in this study. Rice is of particular interest as it can contain DMA and possibly contribute to the association between seafood and DMA. Fourth, the dietary recall was based on self-report, it may have included fresh water fish (usually low in arsenic levels) and it covered the past 24 hours only. Indeed, the results in Table 4 restricted to participants with no seafood intake in the

past 24-h indicate that self-report is an insufficient strategy to remove the contribution of recent seafood intake to urine arsenic concentrations. This can be related to incomplete dietary recall of all seafood sources, potential presence of arsenobetaine, arsenosugars and arsenolipids in unknown sources and the complex metabolism and excretion of organic arsenic compounds. In Hamburg, Germany, urine DMA was similar for participants who ate seafood 0–3 days and 3–6 days before urine collection, and substantially lower only for those participants who ate seafood >6 days ago (Heinrich-Ramm et al. 2001). In certain populations, it can be difficult to eliminate the contribution of organic arsenicals to total urine arsenic and urine DMA by refraining from seafood intake for just a few days.

CONCLUSIONS

In summary, seafood consumption was a major determinant of increased urine concentrations of total arsenic, DMA, arsenobetaine and total arsenic minus arsenobetaine in a representative sample of the general US population including children and adults. These findings highlight the importance of adequately interpreting DMA and total arsenic minus arsenobetaine levels in epidemiologic studies. Most sources of arsenosugars and arsenolipids are also a source of arsenobetaine. In epidemiologic studies, analyses restricted to participants with undetectable arsenobetaine or that adjust for objective markers of seafood intake (e.g. arsenobetaine itself) can thus be used to eliminate the contribution of organic arsenicals from recent seafood intake to total arsenic, DMA and total arsenic minus arsenobetaine concentrations in urine. Based on toxicological experiments and limited epidemiologic evidence, arsenobetaine is not a health concern for the population. For arsenosugars and arsenolipids and their metabolites, including DMA, additional research is needed to evaluate their potential health impact.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AB	Arsenobetaine
BMI	Body mass index
DMA	Dimethylarsinate
HPLC	high performance liquid chromatography
ICP-DRC-MS	Inductively coupled-plasma mass dynamic reaction cell-mass spectrometry
NHANES	National Health and Nutrition Examination Survey
US	United States

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Figure 1.

Contribution of major forms of arsenic in seafood (arsenobetaine, arsenosugars and arsenolipids) to total urine arsenic following recent seafood intake. Given inter-individual differences in arsenobetaine, arsenosugars and arsenolipids excretion patterns, the time frame to define “recent seafood intake” can be different across people. Arrows represent direct contributions. For instance, the frequency of seafood intake in the past year is related to the likelihood of recent seafood intake. Bolded elements correspond to variables that are available in NHANES 2002–2006.

Figure 2.

Relationship of urine arsenobetaine and blood mercury with total arsenic, dimethylarsinate (DMA) and total arsenic minus arsenobetaine in NHANES 2003–2006. Lines represent dose-response relationships based on restricted quadratic spline models that adjusted for urine creatinine concentrations. Correlation coefficients represent spearman correlation coefficients (all are highly statistically significant, $p < 0.001$).

Figure 3. Urine arsenic concentrations by self-reported seafood intake in the past 24-h. Horizontal lines within boxes represent medians; boxes, interquartile range; error bars, values within 1.5 times the interquartile range; circles, outlying data points.

Table 1

Participant characteristics by seafood intake

	Seafood intake past 24-h (N=4276)		Seafood frequency (N=3258)	
	Yes	No	≥1/month	<1/month
N (%)	575 (14.6)	3701 (85.4)	2259 (70.0)	999 (30.0)
Age (yr) - mean (SE)	44.5 (0.9)	39.5 (0.6)	42.1 (0.8)	40.9 (0.7)
Sex - % female (SE)	52.9 (2.6)	49.8 (1.1)	51.5 (1.3)	51.9 (2.0)
Race / ethnicity - % (SE)				
White	67.5 (3.2)	72.2 (2.7)	73.7 (2.5)	74.9 (3.3)
Black	13.8 (1.9)	11.0 (1.4)	10.7 (1.4)	9.9 (1.7)
Mexican-American	6.4 (1.3)	9.1 (1.4)	7.7 (1.3)	7.6 (1.5)
Other	12.3 (2.0)	7.7 (1.0)	7.9 (1.0)	7.7 (1.4)
Education - % (SE) *				
<High school	13.9 (1.5)	18.5 (1.3)	15.8 (1.2)	18.9 (1.8)
High school or equivalent	25.3 (2.8)	27.2 (1.0)	25.7 (1.2)	31.9 (1.9)
>High school	60.8 (2.9)	54.3 (1.5)	58.5 (1.5)	49.2 (2.3)
Body mass index - mean (SE)	27.4 (0.4)	27.1 (0.2)	27.4 (0.2)	26.9 (0.4)
Smoking - % (SE)				
Never smoker	55.5 (3.1)	53.4 (1.4)	55.9 (1.6)	51.2 (3.0)
Former smoker	22.2 (2.0)	19.4 (1.0)	20.5 (1.3)	21.1 (1.8)
Current smoker	22.0 (2.1)	27.2 (1.3)	23.6 (1.5)	27.7 (2.3)
Seafood ≥1/month - % (SE)	82.0 (1.9)	67.9 (1.8)	--	--
Seafood past 24-h - % (SE)	--	--	17.1 (0.9)	8.8 (1.3)
Cotinine (nmol/L)†	0.06 (0.02 – 2.18)	0.09 (0.02 – 13.00)	0.06 (0.02 – 2.04)	0.09 (0.02 – 25.50)
Blood mercury (µg/L) †	1.7 (0.9 – 3.1)	0.8 (0.4 – 1.6)	1.2 (0.6 – 2.2)	0.5 (0.2 – 1.0)
Total urine arsenic (µg/L) †	24.5 (10.1 – 51.2)	7.3 (3.9 – 13.7)	9.0 (4.7 – 19.3)	6.6 (3.4 – 12.2)
Urine DMA (µg/L) †	6.0 (3.3 – 11.3)	3.5 (2.0 – 5.4)	4.0 (2.1 – 6.4)	3.1 (2.0 – 5.1)
Urine arsenobetaine (AB) (µg/L) †	10.2 (4.4 – 28.2)	0.9 (0.3 – 3.7)	1.9 (0.4 – 7.7)	0.5 (0.3 – 2.6)
Total arsenic minus AB (µg/L) †	11.0 (5.0 – 23.5)	5.5 (3.0 – 9.4)	6.0 (3.2 – 11.5)	5.2 (2.7 – 8.9)
% AB detectable - % (SE)	96.5 (1.0)	64.9 (2.0)	76.8 (1.6)	52.7 (2.8)

AB: arsenobetaine; DMA: dimethylarsinate; SE: standard error

* For participants <20 y, education of the household reference person.

† Median (Interquartile range).

Table 2

Urine arsenic concentrations by seafood intake in the past 24-h (N=4276)

	N (%)	Total arsenic	DMA	Arsenobetaine	Total minus arsenobetaine
Geometric mean (95% CI)					
No seafood	3701 (85.4)	7.3 (6.7, 8.0)	3.5 (3.3, 3.7)	1.3 (1.1, 1.4)	5.0 (4.6, 5.5)
Any seafood*	575 (14.6)	24.6 (21.4, 28.3)	6.2 (5.6, 6.9)	10.3 (8.6, 12.5)	10.9 (9.6, 12.3)
Fish	264 (6.5)	26.1 (21.5, 31.7)	6.4 (5.6, 7.3)	10.4 (7.80, 13.8)	11.6 (5.3, 25.5)
Shellfish	124 (3.5)	36.7 (26.2, 51.2)	8.1 (6.4, 10.3)	16.8 (11.4, 24.8)	14.8 (10.7, 20.5)
Mollusks	22 (0.7)	62.5 (32.8, 119)	15.3 (9.5, 24.5)	26.7 (11.7, 60.8)	30.6 (18.9, 52.3)
Sushi/seaweed	18 (0.6)	39.7 (18.2, 86.6)	11.5 (5.6, 23.5)	11.6 (5.26, 25.5)	25.6 (11.2, 58.3)
Ratio of the geometric mean (95% CI) †‡					
No seafood	3700 (85.3)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Any seafood*	576 (14.7)	3.5 (3.1, 3.9)	1.9 (1.7, 2.0)	7.7 (6.5, 9.1)	2.3 (2.1, 2.6)
Fish	264 (6.5)	3.8 (3.1, 4.6)	1.9 (1.7, 2.1)	7.7 (5.6, 10.4)	2.5 (2.2, 2.9)
Shellfish	125 (3.5)	4.9 (3.8, 6.5)	2.3 (2.0, 2.8)	12.1 (8.6, 17.0)	3.0 (2.4, 3.8)
Mollusks	22 (0.7)	7.0 (3.9, 12.5)	3.8 (2.3, 6.4)	15.8 (7.3, 34.3)	5.2 (3.2, 8.3)
Sushi/seaweed	18 (0.6)	5.5 (3.1, 9.5)	3.2 (1.9, 5.5)	9.2 (5.0, 16.9)	5.2 (2.9, 9.2)

* Includes fish or shellfish reported as individual item or in mixture with other food items (e.g. rice, pasta, soup, sandwiches)

Only one person reported seaweed consumption in the past 24-h

† Adjusted for urine creatinine (continuous), age (continuous), sex (men/women), race/ethnicity (white/black/Mexican-American/ Other), education (<high school/ high school or equivalent / >high school), body mass index (continuous), smoking status (never/former/current) and serum cotinine (log-transformed).

‡ All ratios are highly statistically significant (p-values<0.001).

Table 3
Urine arsenic concentrations by frequency of seafood intake in the past year (N=3258)

	N (%)	Total arsenic	DMA	Arsenobetaine	Total minus arsenobetaine
Seafood past year					
Never	223 (5.9)	5.7 (4.8, 6.7)	3.4 (3.1, 3.8)	0.5 (0.4, 0.6)	4.6 (3.9, 5.5)
1–11 times per year	776 (24.1)	6.8 (5.9, 7.8)	3.3 (3.0, 3.6)	1.2 (1.0, 1.4)	4.5 (4.0, 5.2)
1–4 times per month	1806 (57.2)	9.3 (8.5, 10.1)	3.8 (3.6, 4.1)	2.0 (1.8, 2.31)	5.7 (5.3, 6.3)
≥ 2 times per week	453 (12.8)	12.9 (11.1, 14.9)	4.7 (4.2, 5.3)	3.4 (2.8, 4.2)	7.5 (6.6, 8.5)
Ratio of the geometric mean (95% CI) *					
Never	223 (5.9)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1–11 times per year	776 (24.1)	1.2 (1.0, 1.4)	1.0 (0.9, 1.1)	2.1 (1.6, 2.7)	1.0 (0.9, 1.2)
1–4 times per month	1806 (57.2)	1.7 (1.5, 2.0)	1.2 (1.1, 1.3)	3.7 (2.9, 4.6)	1.4 (1.2, 1.6)
≥ 2 times per week	453 (12.8)	2.3 (1.9, 2.7)	1.4 (1.2, 1.6)	6.0 (4.6, 7.8)	1.7 (1.4, 2.0)
p-trend [†]		<0.001	<0.001	<0.001	<0.001

* Adjusted for urine creatinine (continuous), age (continuous), sex (men/women), race/ethnicity (white/black/Mexican-American/ Other), education (<high school/ high school or equivalent / >high school), body mass index (continuous), smoking status (never/former/current) and serum cotinine (log-transformed).

[†] Estimated by adding the frequency of seafood intake as a continuous variable to the model.

Table 4

Ratio (95% CI) * of geometric means of urine arsenic concentrations by frequency of seafood intake using different strategies to remove the contribution of seafood arsenicals to total arsenic and DMA concentrations in urine

	N (%)	Total arsenic	DMA	Arsenobetaine	Total minus arsenobetaine
Analysis restricted to participants without self-reported seafood intake in the past 24-h (N=2821)					
Never	211 (6.5)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1–11 times per year	685 (25.4)	1.2 (1.0, 1.4)	1.0 (0.9, 1.1)	2.0 (1.6, 2.4)	1.0 (0.9, 1.2)
1–4 times per month	1558 (56.4)	1.5 (1.3, 1.8)	1.1 (1.0, 1.3)	3.1 (2.6, 3.8)	1.3 (1.1, 1.5)
≥ 2 times per week	367 (11.6)	1.9 (1.6, 2.3)	1.2 (1.0, 1.4)	5.1 (3.8, 6.8)	1.4 (1.2, 1.7)
p-trend [†]		<0.001	0.002	<0.001	<0.001
Analysis restricted to participants with undetectable arsenobetaine (N= 1391)					
Never	164 (11.4)	1.0 (reference)	1.0 (reference)	--	--
1–11 times per year	312 (25.2)	0.93 (0.81, 1.06)	0.92 (0.83, 1.01)	--	--
1–4 times per month	500 (36.9)	1.02 (0.88, 1.19)	0.95 (0.83, 1.07)	--	--
≥ 2 times per week	85 (5.0)	1.01 (0.86, 1.19)	0.89 (0.75, 1.05)	--	--
p-trend [†]		0.34	0.42		
Analysis in the overall study sample with further adjustment for urine arsenobetaine concentrations (N=3258)					
Never	223 (5.9)	1.0 (reference)	1.0 (reference)	--	1.0 (reference)
1–11 times per year	776 (24.1)	0.85 (0.76, 0.96)	0.89 (0.82, 0.97)	--	0.87 (0.76, 1.00)
1–4 times per month	1806 (57.2)	0.91 (0.80, 1.02)	0.96 (0.86, 1.07)	--	0.98 (0.85, 1.14)
≥ 2 times per week	453 (12.8)	0.94 (0.82, 1.07)	1.04 (0.91, 1.19)	--	1.06 (0.91, 1.23)
p-trend [†]		0.53	0.06		0.04

* Adjusted for urine creatinine (continuous), age (continuous), sex (men/women), race/ethnicity (white/black/Mexican-American/ Other), education (<high school/ high school or equivalent / >high school), body mass index (continuous), smoking status (never/former/current) and serum cotinine (log-transformed).

[†] Estimated by adding the frequency of seafood intake as a continuous variable to the model.