Low-level Population Exposure to Inorganic Arsenic in the United States and Diabetes Mellitus

A Reanalysis

Craig Steinmaus,[a,b] *Yan Yuan,*[b] *Jane Liaw,*[b] *and Allan H. Smith*[b]

Background: Although studies have reported associations between high concentrations of ingested inorganic arsenic and diabetes mellitus, there is no evidence of this association at low exposures. However, a well-publicized study (*JAMA*. 2008;300:814–822) recently produced an extraordinary finding of a more than 3-fold increase in diabetes at low concentrations of urinary arsenic. This potentially affects 40 million adults in the United States.

Methods: We used the same cross-sectional data on urinary arsenic and type 2 diabetes mellitus in 795 adults from the 2003–2004 National Health and Nutrition Examination Survey to assess this evidence.

Results: As in the earlier study, we found an odds ratio (OR) near 1.0 for diabetes, comparing the 80th versus 20th percentiles of urinary total arsenic (OR = 0.88 [95% confidence interval = 0.39–1.97]). This OR increased to above 3.0 when urinary arsenobetaine was added to the logistic risk model. However, this high OR was a statistical artifact because arsenobetaine, which is ingested from fish and is essentially nontoxic, is a part of measured total urinary arsenic. These 2 variables are highly correlated (correlation = 0.80). Because arsenobetaine is a part of total arsenic, it should first be subtracted from total arsenic rather than being added to the statistical model. Doing so yields an OR of 1.15 (0.53–2.50).

Conclusion: These findings show no evidence of increased risk of diabetes with arsenic exposure in this dataset. This underscores the importance of valid statistical techniques and careful consideration of scientific plausibility when investigating low-concentration chemical exposures.

(*Epidemiology* 2009;20: 807–815)

Submitted 2 February 2009; accepted 1 May 2009.

From the [a]California Environmental Protection Agency, OEHHA; and [b]Arsenic Health Effects Research Program, University of California, Berkeley.

Supported by the National Institute of Environmental Health Sciences (NIEHS) grant P42-ES04705 and the University of California Center of Occupational and Environmental Health (COEH).

Editors' note: *Related articles appear on pages 816 and 821.*

SDC Supplemental digital content is available through direct URL citations in the HTML and PDF versions of this article (www.epidem.com).

Correspondence: Craig Steinmaus, Arsenic Health Effects Research Program, School of Public Health, University of California, 50 University Hall, Berkeley, CA 94720. E-mail: craigs@berkeley.edu.

ISSN: 1044-3983/09/2006-0807
DOI: 10.1097/EDE.0b013e3181b0fd29

Arsenic is found in the environment in 2 general forms: inorganic and organic. Inorganic arsenic causes cancers of the lung, bladder, and skin, as well as skin lesions, cardiovascular diseases, and reproductive effects.[1] Tens of millions of people worldwide are exposed to inorganic arsenic, mostly from naturally contaminated drinking water.[2] In the United States, the Environmental Protection Agency has estimated that 25% of all public water systems have arsenic concentrations between 5 and 10 μg/L, with most of the remaining systems having lower concentrations.[3] Inorganic arsenic in the environment exists in 2 different valence states: arsenous acid and arsenic acid. The metabolic pathway of ingested arsenous acid and arsenic acid in humans is methylation, first to monomethylarsonic acid and then to dimethylarsonic acid,[4] although the ability of people to do this varies widely.[5] Inorganic arsenic and its metabolites are excreted in the urine, and the sum of their urinary concentrations is commonly used to assess inorganic arsenic exposure.[1]

Exposure to organic arsenic comes primarily through the consumption of seafood, and these forms are rapidly excreted and believed to be nontoxic.[6,7] The most common form of organic arsenic found in seafood and in human urine is arsenobetaine, although other organic arsenic compounds (including arsenocholine and other arsenosugars) are also present.

Several studies in populations exposed to high concentrations of inorganic arsenic in drinking water (mostly above 500 μg/L) have reported associations between these exposures and type 2 diabetes mellitus (eTable; http://links.lww.com/EDE/A333).[8] Currently, there is no clear evidence that this association also occurs at lower concentrations such as those commonly found in the United States. Recently, a published study reported an odds ratio (OR) for type 2 diabetes mellitus of 3.58 (95% confidence interval [CI] = 1.18 –10.8) for United States subjects at the 80th percentile of urinary total arsenic concentrations compared with those at the 20th percentile.[9] Importantly, this study involved subjects from the 2003–2004 National Health and Nutrition Examination Survey (NHANES), which is designed to provide a nationally representative sample of all noninstitutionalized people in the United States. This finding, if true, would be extraordinary because it would suggest that over 40 million adults in this

country (those in the highest 20% of exposure) have a more than 3-fold increased risk of this highly prevalent and serious disease due to arsenic exposure. This finding was reported by major news outlets, including CNN, US News and World Report, and *USA Today*.[10–12] However, if this result is not correct, then scientific attention, research resources, and public health concern could be inappropriately diverted from other, more important issues.

Analyses of the health effects of inorganic arsenic using data from NHANES 2003–2004 are complicated by the fact that most subjects have very low urinary levels of inorganic arsenic. Another complicating factor is the presence of urinary arsenobetaine and other nontoxic organic forms of arsenic. In the aforementioned analysis of the NHANES 2003–2004 data,[9] these issues were addressed by removing people with recent seafood consumption, and by calculating ORs for type 2 diabetes mellitus and total urinary arsenic (organic + inorganic forms) using logistic regression models that included arsenobetaine and mercury (another indicator of seafood consumption). This method could lead to an important error: arsenobetaine is a part of total arsenic and these 2 variables are highly correlated. Entering 2 highly correlated variables into the same regression model can cause unstable results and lead to incorrect conclusions.[13–15] To avoid this problem, we obtained an estimate of inorganic arsenic exposure by subtracting arsenobetaine from total arsenic. This method directly removes any effects that may be caused by the most common form of organic arsenic. It also prevents aberrant findings that may result from entering both total arsenic and one of its major components (arsenobetaine) into the same logistic regression model.

METHODS

Study Population

The data for this study were obtained from NHANES 2003–2004, which is a national survey conducted by the Centers for Disease Control and Prevention and involving 10,122 people who were selected using a multistage probability sampling design.[16] In NHANES 2003–2004, urinary levels of total arsenic and various forms of inorganic and organic arsenic were analyzed in a representative one-third subsample (n = 2673) of subjects aged 6 years and older. The NHANES 2003–2004 study was approved by the National Center for Health Statistics Institutional Review Board.

Exclusion Criteria

For our study, the following exclusion criteria were applied. First, since our focus was on type 2 diabetes mellitus (which more commonly affects adults) we excluded 1062 subjects under the age of 20. Second, we excluded 584 subjects who did not fast for 8–24 hours before blood collection, 38 women who were pregnant, and 34 subjects missing arsenic speciation data. Third, we excluded 24 subjects without serum glucose measurements and without a self-reported diagnosis of type 2 diabetes mellitus. Fourth, we excluded 133 subjects who reported seafood consumption in their first-day dietary survey, to reduce the contribution of organic arsenic in fish to total urine arsenic concentrations. Three subjects who had urinary arsenobetaine levels greater than total arsenic levels were excluded as this would have caused negative values for our estimates of inorganic arsenic. Finally, in the adjusted logistic regression analysis, 12 additional people were excluded because of missing data on covariates. These are the same exclusion criteria as in the previous analysis[9] except that we did not remove the 4 subjects with missing data on glycated hemoglobin (because we did not analyze this variable), but we removed 3 additional people with seafood consumption due to slight differences in how seafood was defined.

Laboratory Analysis

The methods used to collect and perform all laboratory analyses are detailed elsewhere.[17] Briefly, spot urine samples were collected from all subjects, and shipped on dry ice and stored frozen at −70°C.[18] Total urine arsenic concentrations were measured at the National Center for Environmental Health, using inductively coupled-plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS) on a PerkinElmer ELAN DRC Plus or ELAN DRC II ICP-MS (PerkinElmer SCIEX, Concord, ON, Canada). The concentrations of speciated arsenic were determined using high-performance liquid chromatography to separate the species and ICP-DRC-MS for detection. The interassay coefficient of variation for bench quality control samples run throughout the study was 9.2% for total arsenic for lots with a mean total arsenic of 8.15 μg/L. The interassay coefficients of variation for bench quality control samples with a mean arsenobetaine of 4.87 μg/L and samples with a mean dimethylarsonic acid of 6.66 μg/L were 10.1% and 6.5%, respectively.[19,20] Detection limits in μg/L were 0.6 for total arsenic, 1.2 for arsenous acid, 1.0 for arsenic acid, 0.4 for arsenobetaine, 0.6 for arsenocholine, 1.7 for dimethylarsonic acid, and 0.9 for monomethylarsonic acid. Urinary creatinine concentrations were measured using a Jaffé rate reaction and a CX3 analyzer (Beckman Instruments Inc, Brea, CA). Serum cotinine was measured using an isotope-dilution-high-performance liquid chromatography or atmospheric pressure chemical ionization tandem mass spectrometric method, and serum glucose was measured using a Beckman Synchron LX20 (Beckman Coulter Inc, Fullerton, CA).

Other Data

NHANES questionnaire data included self-reported information on sociodemographic variables and on medical and smoking history. Diet information was collected using a 24-hour dietary recall, and seafood was defined as any food item containing fish or shellfish. We defined people with type

2 diabetes mellitus as those who reported they had been told by a health care professional that they had diabetes or who reported taking insulin or diabetic pills. Subjects who had a fasting serum glucose greater than 125 mg/dL were also defined as having type 2 diabetes mellitus.[21] This was the same definition used in the earlier report.[9]

Statistical Analysis

Because many subjects had levels of arsenous acid, arsenic acid, and monomethylarsonic acid below detection limits, the concentration of inorganic arsenic and its metabolites was estimated by subtracting the concentration of the measured forms of organic arsenic (arsenobetaine and arsenocholine) from the concentration of total arsenic. Earlier studies have shown that arsenobetaine makes up the majority of organic arsenic in most common seafood (the primary source of human exposure) and the majority of organic arsenic commonly found in human urine.[6,7,22]

Initially, analyses were conducted of the relationship between diabetes mellitus and individual variables, including total arsenic, arsenobetaine, dimethylarsonic acid, estimated inorganic arsenic, and other factors that might be related to diabetes or arsenic exposure. Similar analyses were done to assess the association between these variables and other factors that may be related to diabetes or arsenic exposure. Using logistic regression, we calculated odds ratios (ORs) and their 95% confidence intervals (CIs) for type 2 diabetes mellitus and total arsenic, arsenobetaine, dimethylarsonic acid, and estimated inorganic arsenic. Variables were not logarithm transformed because logistic regression does not require that the independent variables be normally distributed, and logarithmic values may distort dose-response relationships. As is standard in NHANES, the concentrations of total arsenic and arsenobetaine below the detection limit were assigned a value of the detection limit divided by the square root of two. Assigning these a value of zero had little impact on our results. Only 13 people had detectable levels of arsenocholine, and so levels below detection were assigned a value of zero. We calculated unadjusted ORs and also ORs adjusted for potential confounding variables. These variables included age (years), sex, race (non-Hispanic white, non-Hispanic black, Mexican-American, other), education (<high school, finished high school, >high school), body mass index (BMI; kg/m^2), serum cotinine (ng/mL), urine albumin (μg/mL), blood mercury (μg/L), urinary creatinine (mg/dl), and the use of blood pressure medications (current use self-reported as yes or no). We chose these variables because they have been associated with the risks of diabetes or other arsenic-related diseases, or with urinary concentrations of glucose or arsenic in other studies.[1,23] Age, BMI, blood mercury, urinary creatinine, urinary albumin, and serum cotinine were entered as continuous variables. Entering these as categorical variables had little impact on results. Blood mercury and urine albumin had little effect and were removed from the final analyses. In the past, dividing urinary arsenic concentration by urinary creatinine concentration was a common way of adjusting for urine dilution.[24] However, this can have undesirable consequences, especially if creatinine is related to the outcome of interest. Because patients with diabetes have lower creatinine levels than nondiabetics,[24] we did not use this adjustment. We initially entered creatinine as a continuous variable in the logistic regression models but did not include it in the final results since it had little effect on our findings. This is consistent with several previous studies showing that creatinine adjustment does not improve arsenic exposure estimates[25–27] (creatinine-adjusted results are available at: http://asrg.berkeley.edu/research.html).

Odds ratios for the associations between type 2 diabetes mellitus and total arsenic, arsenobetaine, dimethylarsonic acid, and estimated inorganic arsenic were calculated for each tertile or quintile using the lowest tertile or quintile as the reference. The previous analysis of these data[9] calculated odds ratios using model-predicted odds at specific urinary arsenic levels. For example, the odds ratio for the 80th versus 20th percentiles was calculated as the odds predicted for a urine arsenic level at the 80th percentile cut-off point divided by the odds predicted for an arsenic level at the 20th percentile cut-off point. We chose to base our comparison on actual arsenic exposure categories (eg, we compare the odds in all subjects above the 80th percentile to the odds in all subjects below the 20th percentile) because this allows presentation of easily interpreted 2 $\times$ 2 table data and the development of odds ratios based on actual data rather than model predictions.

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC), and were done with and without incorporating the NHANES sampling weights. These weights are used to produce unbiased national estimates, and reflect the unequal probabilities of selection, nonresponse adjustments, and adjustments to independent population controls.[28] These weights had little effect on our results. The results we present here are calculated without weights, because our goal was not to produce nationally representative estimates of population characteristics but rather to assess a possible causal association.

RESULTS

Of the 2673 people with urine arsenic data in NHANES 2003–2004, 795 people met our inclusion criteria. Of these, a total of 98 met our criteria for type 2 diabetes mellitus: 77 had a self-reported diagnosis of diabetes and 21 were categorized as having diabetes based on their fasting serum glucose measurement. Of the 795 subjects, the percentage with levels below detection were: arsenous acid, 96%; arsenic acid, 93%; dimethylarsonic acid, 15%; monomethylarsonic acid, 65%; arsenobetaine, 33%; and arsenocholine, 98%.

Table 1 shows the number of people with and without type 2 diabetes mellitus, and the means of the forms of

TABLE 1. Sociodemographic Variables and Urine Arsenic Levels (μg/L) in the Study Participants

	Diabetes		Total Arsenic		Organic Arsenic[a]		Inorganic Arsenic[b]	
	Yes No. (%)	No No. (%)	Median	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)
All	98 (12.3)	697 (87.7)	7.6	16.7 (39.7)	1.0	7.3 (31.7)	6.0	9.4 (12.9)
Age (years)								
20–39	5 (5.1)	254 (36.4)	9.0	18.2 (52.2)	1.0	7.6 (44.3)	6.7	10.6 (14.1)
40–59	26 (26.5)	217 (31.1)	7.4	17.3 (38.8)	1.1	7.8 (29.1)	5.7	9.4 (14.3)
≥60	67 (68.4)	226 (32.4)	7.1	15.0 (25.2)	0.9	6.5 (17.4)	5.3	8.5 (10.3)
Sex								
Male	55 (56.1)	364 (52.2)	9.1	17.7 (29.2)	1.1	6.8 (19.8)	6.8	10.9 (14.5)
Female	43 (43.9)	333 (47.8)	6.5	15.7 (48.9)	0.8	7.8 (41.2)	5.1	7.9 (10.7)
BMI (kg/m^2)								
<25	16 (16.7)	227 (32.9)	6.9	18.4 (55.4)	0.8	8.9 (46.8)	5.7	9.5 (13.8)
25–<30	30 (31.2)	261 (37.8)	8.4	18.7 (38.4)	1.0	8.4 (28.5)	6.6	10.3 (14.7)
≥30	50 (52.1)	202 (29.3)	7.6	13.0 (17.6)	1.1	4.5 (10.9)	5.6	8.5 (9.6)
Race/ethnicity								
White	43 (43.9)	376 (54.0)	6.1	14.2 (46.0)	0.8	7.0 (38.9)	4.9	7.2 (10.0)
Black	19 (19.4)	154 (22.1)	9.2	17.9 (26.6)	1.7	7.3 (17.7)	6.6	10.7 (11.6)
Mexican-American	33 (33.7)	128 (18.4)	9.3	17.2 (31.9)	0.7	6.3 (23.7)	7.7	10.9 (14.1)
Other	3 (3.1)	39 (5.6)	18.7	35.5 (40.7)	3.5	13.9 (22.5)	13.0	21.6 (25.7)
Type 2 diabetes mellitus								
Yes			7.5	16.0 (27.0)	0.7	4.9 (13.1)	6.2	11.1 (16.9)
No			7.6	16.8 (41.2)	1.0	7.6 (33.5)	6.0	9.2 (12.3)
Serum cotinine (ng/mL)								
Nondetectable	26 (27.4)	120 (17.2)	6.8	13.4 (25.3)	0.8	5.9 (18.9)	5.7	7.5 (7.6)
0.015–10	46 (48.4)	384 (55.1)	7.7	18.3 (45.8)	1.1	8.3 (37.9)	6.0	10.0 (13.7)
≥10	23 (24.2)	193 (27.7)	7.9	15.8 (34.7)	0.9	6.2 (24.6)	6.3	9.6 (14.0)
Blood pressure medication								
Yes	55 (56.1)	151 (21.7)	7.4	17.1 (39.9)	1.3	8.5 (31.2)	5.4	8.6 (11.3)
No	43 (43.9)	546 (78.3)	7.6	16.6 (39.7)	0.9	6.8 (31.9)	6.1	9.7 (13.4)
Education								
<High school	46 (46.9)	196 (28.1)	7.6	15.4 (25.1)	0.7	5.3 (15.0)	6.4	10.1 (13.5)
High school	12 (12.2)	204 (29.3)	8.6	18.0 (38.3)	1.2	8.5 (29.7)	6.3	9.5 (13.1)
>High school	40 (40.8)	297 (42.6)	7.2	16.9 (48.4)	1.0	7.9 (40.6)	5.7	9.0 (12.4)

[a]Organic arsenic is the sum of arsenobetaine and arsenocholine. Arsenocholine is included only if it is above the detection limit.
[b]Inorganic arsenic is total arsenic minus organic arsenic.

arsenic by age, sex, race, education, BMI, use of blood pressure medication, and serum cotinine. The prevalence of diabetes mellitus was associated with current use of blood pressure medication (crude OR = 4.6 [95% CI = 3.0–7.2]) and BMI (crude OR for BMI ≥30 vs. <25, 3.5 [1.9–6.4]).

The odds ratios for type 2 diabetes mellitus comparing people above the upper 80th percentile of each arsenic form with subjects below the 20th percentile, and comparing people in the upper and middle tertiles with those in the lower tertiles, are shown in Table 2. For total arsenic, the adjusted odds ratio for type 2 diabetes mellitus for those above the 80th percentile compared with those below the 20th percentile was 0.88 (95% CI, 0.39 –1.97). Entering the logarithm of urinary arsenobetaine and creatinine into this model with total arsenic, as done by Navas-Acien et al,[9] produced an odds ratio for diabetes above 3.0 (Table 3). However, this large increase is due to arsenobetaine being part of total urinary arsenic, which results in collinearity from a strong correlation between total arsenic and arsenobetaine. The Spearman and Pearson correlation coefficients between urinary levels of total arsenic and arsenobetaine were 0.71 and 0.96, respectively. The Figure provides a plot of these variables, illustrating the strong correlation between them. After adjusting for urinary creatinine, the Pearson correlation coefficient was 0.80 between total arsenic and arsenobetaine (both log-transformed) and 0.49 between inorganic arsenic and arsenobetaine (both log-transformed). The corresponding R^2 values were 0.64 and 0.24, respectively.

For our estimate of inorganic arsenic, the adjusted odds ratio for type 2 diabetes mellitus for those above the 80th

TABLE 2. Associations of Urine Arsenic Concentrations With Type 2 Diabetes Mellitus

	No. Cases	No. Controls	Unadjusted OR (95% CI)	Adjusted[a] OR (95% CI)
Total arsenic				
Percentile				
≤20th (≤3.5 μg/L)[b]	21	141	1.00	1.00
≥80th (≥18.3 μg/L)	17	142	0.80 (0.41–1.59)	0.88 (0.39–1.97)
Tertile				
Lower (≤5.2 μg/L)[b]	36	232	1.00	1.00
Middle (5.3–11.8 μg/L)	32	230	0.90 (0.54–1.49)	0.87 (0.48–1.55)
Upper (>11.8 μg/L)	30	235	0.82 (0.49–1.38)	0.76 (0.42–1.39)
Arsenobetaine				
Percentile				
Below detection[c] (≤0.3 μg/L)[b]	42	217	1.00	1.00
≥80th (≥6.2 μg/L)	15	142	0.55 (0.29–1.02)	0.59 (0.29–1.19)
Tertile				
Lower (≤0.3 μg/L)[b]	42	217	1.00	1.00
Middle (0.4–2.2 μg/L)	29	246	0.61 (0.37–1.01)	0.68 (0.38–1.21)
Upper (>2.2 μg/L)	27	234	0.60 (0.36–1.00)	0.62 (0.34–1.13)
Estimated inorganic arsenic				
Percentile				
≤20th (≤2.7 μg/L)[b]	20	142	1.00	1.00
≥80th (≥11.9 μg/L)	22	139	1.12 (0.59–2.15)	1.15 (0.53–2.50)
Tertile				
Lower (≤4.1 μg/L)[b]	36	227	1.00	1.00
Middle (4.2–8.5 μg/L)	29	238	0.77 (0.46–1.30)	0.63 (0.34–1.15)
Upper (>8.5 μg/L)	33	232	0.90 (0.54–1.49)	0.98 (0.53–1.80)
Dimethylarsinic acid (DMA)				
Percentile				
≤20th (≤1.9 μg/L)[b,d]	15	111	1.00	1.00
≥80th (≥7.0 μg/L)	21	146	1.06 (0.53–2.16)	1.05 (0.45–2.44)
Tertile				
Lower (≤2.7 μg/L)	35	227	1.00	1.00
Middle (2.8–5.0 μg/L)	37	252	0.95 (0.58–1.56)	1.02 (0.57–1.82)
Upper (>5.0 μg/L)	26	218	0.77 (0.45–1.33)	0.82 (0.43–1.57)

[a]Adjusted for sex, age, ethnicity, education, body mass index, serum cotinine, and current use of hypertension medications.
[b]Reference category.
[c]The detection limit was used rather than the 20th percentile since >20% of people had arsenobetaine concentrations below detection.
[d]This group contains fewer than 159 subjects (the 20th percentile) since a large number of subjects had DMA levels of 2 μg/L and were assigned to the 20th–80th percentile group.

percentile compared with those below the 20th percentile was 1.15 (0.53–2.50) (Table 2). No clear trend was seen in the analysis involving tertiles. For dimethylarsonic acid (the most common form of inorganic arsenic) the type 2 diabetes mellitus odds ratio comparing subjects above the 80th percentile with those below the 20th percentile was 1.05 (0.45–2.44).

The differences between our results and those of the previous analysis of these data are shown in Table 3. When we repeated the analyses using the same methods used by Navas-Acien et al,[9] we obtained results similar to theirs. For example, the diabetes odds ratio comparing the model-predicted odds at the 80th and 20th percentiles of total arsenic adjusted for the logarithms of arsenobetaine, blood mercury, and urine creatinine was 3.58 (1.18–10.83) in their analysis and 3.57 (1.28–9.95) in ours. The corresponding odds ratios without arsenobetaine and mercury were 1.05 (0.57–1.94) in their analysis and 1.06 (0.61–1.86) in ours. The minor differences are probably due to differences in the statistical programs (*R* vs. SAS) and the fact that we did not exactly replicate the number of people they removed due to recent seafood consumption (we removed 133 and they removed 130).

DISCUSSION

Our results suggest no identifiable association between type 2 diabetes mellitus and urinary inorganic arsenic in the general United States population using NHANES data. We computed an odds ratio for diabetes mellitus of 1.15 (0.53–2.50) for subjects with estimated urinary inorganic arsenic levels above the 80th percentile compared with those with levels below the 20th percentile, and similar odds ratios near

TABLE 3. Associations of Urine Arsenic Concentrations With Type 2 Diabetes Mellitus: Comparison of Our Results With Those of Navas-Acien et al[9]

	Total Arsenic OR (95% CI)	After Adjusting for Arsenobetaine[a] OR (95% CI)
Our findings[b]	0.88 (0.39–1.97)	1.15 (0.53–2.50)
Navas-Acien et al[c]	1.05 (0.57–1.94)	3.58 (1.18–10.83)
Our replication of Navas-Acien et al[c,d]	1.06 (0.61–1.86)	3.57 (1.28–9.95)

[a]For our findings, arsenobetaine is subtracted from total arsenic. In Navas-Acien et al,[9] and in our replication of the Navas-Acien et al methods, arsenobetaine and total arsenic are both included as independent variables in the same logistic regression model.
[b]The diabetes odds ratio comparing subjects above the 80th percentile to those below the 20th percentile.
[c]The diabetes odds ratio comparing the model-estimated odds in subjects at the 80th percentile to the model-estimated odds in subjects at the 20th percentile.
[d]The results of our analysis using the same methods used by Navas-Acien et al.

1.0 in our analysis of estimated inorganic arsenic tertiles. These findings are not unexpected, given the very low arsenic levels seen in the large majority of the NHANES subjects.

The clearest evidence linking inorganic arsenic in drinking water to diabetes risks in previous studies comes from investigations in which many people were exposed to arsenic concentrations above 500 μg/L. These levels are about 10–30 times higher than those found in the top 20th percentile of the 2003–2004 NHANES subjects.[29–35] (A supplemental table reviewing this previous research is available at: http://asrg.berkeley.edu/.) The relatively modest relative risks seen in these high exposure studies suggest that if arsenic did increase the risk of diabetes mellitus at common United States exposure levels (ie, water concentrations less than 10 μg/L), this effect would be undetectable in epidemiologic studies. Identifying associations where relative risks are expected to be low requires very large studies for adequate statistical power. It also requires very accurate data on exposure and potential confounders, which are more likely to have important effects on study results and conclusions when relative risks are low than when they are high.[36] Because of these issues, identifying an association between arsenic and diabetes mellitus in a general low-exposure population is likely not possible, even if the association were real.

One potential problem in using NHANES 2003–2004 data to investigate the health effects of arsenic is that only a single spot urine sample was collected from each subject. Urinary arsenic levels can vary over time within an individual if that person consumes water from different sources with different arsenic concentrations. This is especially important in diseases such as diabetes, which may take years to develop. The use of a single urine sample would lead to misclassification of arsenic exposure in some subjects. Similar bias could also occur from inaccuracies in diagnosing diabetes, including errors in self-reported diagnoses or day-to-day variability in serum glucose. Because information about diabetes and arsenic exposure were collected similarly in all individuals, the resulting bias from these factors is most likely nondifferential and thus toward the null. The use of cross-sectional data also raises questions about the temporal relationship between diabetes and urinary arsenic, although we are not aware of any data suggesting that diabetes causes a change in arsenic excretion. In contrast, past studies linking high levels of arsenic and diabetes, in which arsenic exposure was estimated mostly by using drinking water concentrations rather than urine, provide a fairly substantial body of evidence for the temporal relationship of high levels of arsenic ingestion resulting in an increased prevalence of diabetes.

Inaccuracies may have also been introduced by the fact that many subjects had levels of inorganic arsenic metabolites below detection. It is possible that our method of subtracting arsenobetaine and arsenocholine from total arsenic to estimate inorganic arsenic did not remove all forms of organic arsenic in some individuals. However, most evidence suggests that the remaining amount of organic arsenic is likely to be small. Some types of seafood contain arsenosugars that are distinct from arsenobetaine and arsenocholine, and some of these are metabolized to dimethylarsonic acid in humans, but these types of seafood (mussels, clams, seaweed, algae) are likely eaten only rarely and only by a relatively small percentage of the general NHANES population. Thus, the effect of these on our results is likely to be small. Most evidence suggests that arsenobetaine accounts for most of the organic arsenic in food.[7,22,37–40] These data also show that arsenobetaine is excreted mostly unchanged in human urine and is the most common organic form of arsenic in background samples of human urine.

We assessed the possibility that our estimate of inorganic arsenic was inaccurate due to the presence of unaccounted-for forms of organic arsenic, by calculating the percentage of total arsenic that was present as arsenous acid, arsenic acid, monomethylarsonic acid, dimethylarsonic acid, trimethylarsine oxide, arsenobetaine, and arsenocholine. Initially, in subjects who had levels of these metabolites below detection, we assigned values that were at the level of detection divided by the square root of 2. When this was done, the sum of the individual inorganic and organic metabolites was on average greater than 100% of the concentration of total arsenic. If measurements below detection are set at zero, this percentage averaged 75% (52% inorganic forms and 23% organic forms).

The finding that arsenobetaine adjustment causes a large change in the diabetes OR for total arsenic is consistent with the R^2 values for these variables. The R^2 values we calculated show that arsenobetaine accounts for 64% of the variance in total arsenic but only 24% of the variance in our estimate of inorganic arsenic. The correlation between our estimate of inorganic arsenic and arsenobetaine is probably

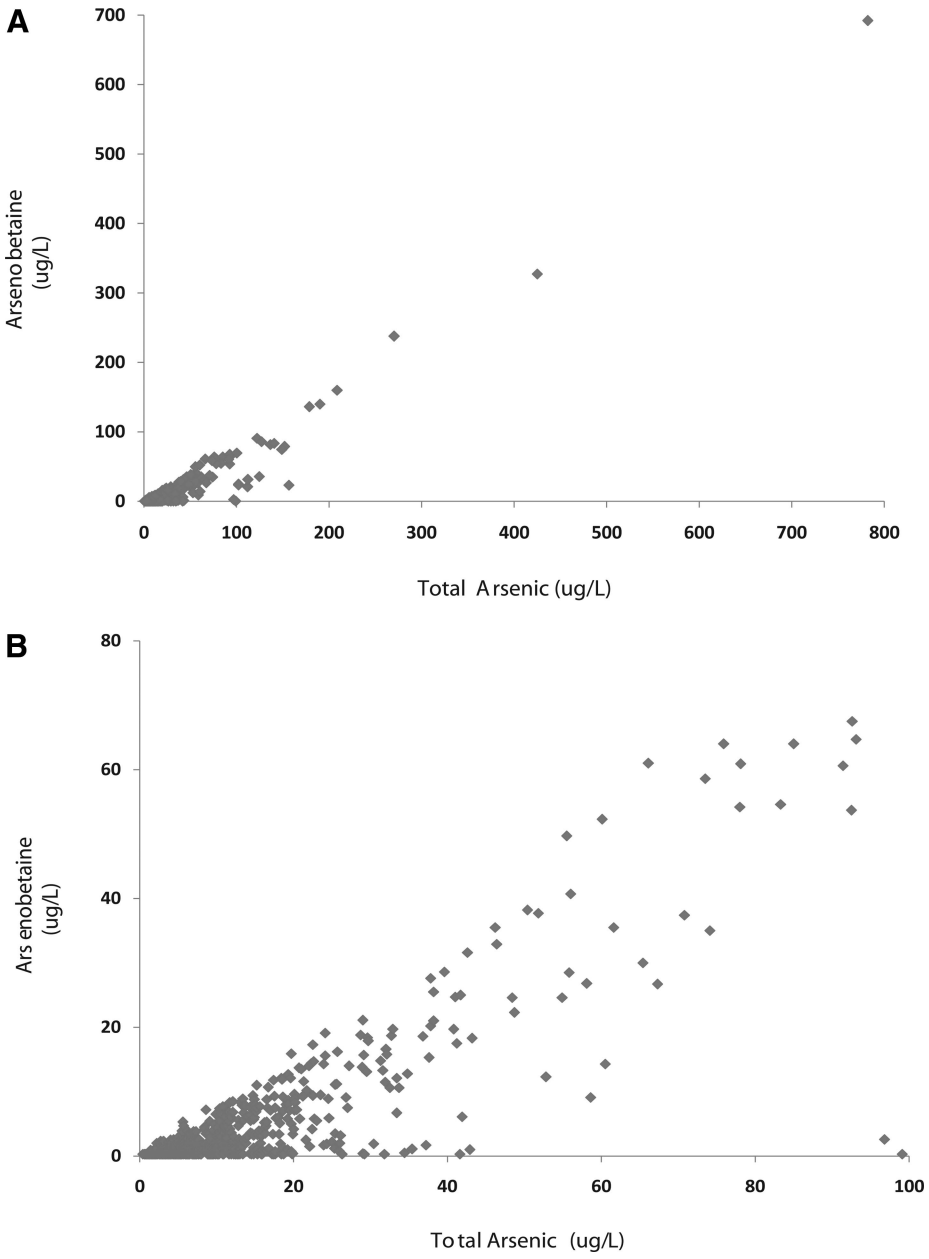

FIGURE. Plot of the correlation between urinary total arsenic and arsenobentaine.

due to some dimethylarsonic acid and some other arsenosugars that are present in the same seafoods that contain arsenobetaine. When we entered total arsenic and arsenobetaine into the same model used in the previous analysis of these data, we obtained a diabetes odds ratio of 3.57 comparing the 80th to 20th percentiles of total arsenic—virtually the same as the OR of 3.58 obtained by Navas-Acien et al (our Table 3). However, the strong correlation between total arsenic and arsenobetaine, and our findings of odds ratios near 1.0 when arsenobetaine was subtracted from total arsenic, show that this high odds ratio is an artifact resulting from incorporating both total urinary arsenic and arsenobetaine (which is part of total urinary arsenic) into the same mathematical model. A correct analysis of the data shows there is no evidence of increased diabetes prevalence with increasing urinary inorganic arsenic in this dataset.

In summary, our analysis did not produce evidence that the low levels of arsenic exposure commonly found in the United States are associated with type 2 diabetes mellitus. This result is consistent with previous studies on arsenic and diabetes, which show mostly modest increases in relative risk at exposure levels that are much higher than found in our study. The greater than 3-fold increase in risk seen in the previous analysis of the NHANES dataset resulted from

inappropriately entering arsenobetaine into the same logistic risk model with total urinary arsenic (which includes arsenobetaine). Our findings and those of Navas-Acien et al illustrate the difficulties encountered when investigating low exposure levels commonly seen in large population surveys such as NHANES. This paper underscores the importance of careful statistical analyses and a thorough consideration of scientific plausibility and bias when planning or conducting investigations of very low chemical exposures. Accurately evaluating type 2 diabetes mellitus at exposure levels in the general United States population would likely have to involve unusually large and prohibitively expensive studies. Given this, and given that the evidence linking arsenic exposures above 200 μg/L to diabetes mellitus is fairly strong, the next logical step for investigating diabetes-arsenic relationships epidemiologically would be to focus on moderate exposure levels (eg, about 50–200 μg/L in drinking water). Studies of arsenic below the current drinking water standard of 10 μg/L (experienced by most of the United States population) would be warranted if clearly increased risks could first be demonstrated in populations exposed to water containing arsenic concentrations of 50–200 μg/L.

ACKNOWLEDGMENTS

Chiron Alston assisted in the editing and preparation of this paper.

REFERENCES

1. NRC. *Arsenic in Drinking Water 2001 Update, Subcommittee to Update the 1999 Arsenic in Drinking Water Report*. Washington, DC: National Research Council; 2001.
2. Nordstrom DK. Public health. Worldwide occurrences of arsenic in ground water. *Science*. 2002;296:2143–2145.
3. USEPA. *Arsenic Occurrence in Public Drinking Water Supplies*. Washington, DC: US Environmental Protection Agency; 2000.
4. Styblo M, Drobna Z, Jaspers I, Lin S, Thomas DJ. The role of biomethylation in toxicity and carcinogenicity of arsenic: a research update. *Environ Health Perspect*. 2002;110(suppl 5):767–771.
5. Loffredo CA, Aposhian HV, Cebrian ME, Yamauchi H, Silbergeld EK. Variability in human metabolism of arsenic. *Environ Res*. 2003;92:85–91.
6. Cullen WR, Reimer KJ. Arsenic speciation in the environment. *Chem Rev*. 1989;89:713–764.
7. Edmonds J, Francesconi K. Arsenic in seafoods: human health aspects and regulations. *Mar Pollut Bull*. 1993;26:665–674.
8. Chen CJ, Wang SL, Chiou JM, et al. Arsenic and diabetes and hypertension in human populations: a review. *Toxicol Appl Pharmacol*. 2007;222:298–304.
9. Navas-Acien A, Silbergeld EK, Pastor-Barriuso R, Guallar E. Arsenic exposure and prevalence of type 2 diabetes in US adults. *JAMA*. 2008;300:814–822.
10. Brophy Marcus M. Inorganic arsenic in water may be linked with diabetes risk. *USA Today*. August 20, 2008. Available at: http://www.usatoday.com/news/health/2008-08-19-arsenic-diabetes_N.htm. Accessed August 19, 2008.
11. CNN Study. Possible diabetes link to arsenic in water. Available at: http://www.cnn.com/2008/HEALTH/conditions/08/19/arsenic.diabetes.ap/index.html#cnnSTCText. Accessed October 26, 2008.
12. Voiland A. Is your drinking water giving you diabetes? Arsenic, a common trace contaminant in well water, has been linked to type 2 diabetes. US News and World Report. August 19, 2008. Available at: http://health.usnews.com/articles/health/diabetes/2008/08/19/is-your-drinking-water-giving-you-diabetes.html. Accessed August 19, 2008.
13. Jewell NP. Statistics for Epidemiology. In: Chatfield C, Tanner M, Zidek J, eds. *Texts in Statistical Science*. Boca Raton, FL: Chapman and Hall/CRC; 2004:230–233.
14. Tu YK, Clerehugh V, Gilthorpe MS. Collinearity in linear regression is a serious problem in oral health research. *Eur J Oral Sci*. 2004;112:389–397.
15. Clayton D, Hill M. *Statistical Models in Epidemiology*. Oxford: Oxford University Press; 2005:247.
16. National Center for Health Statistics. Centers for Disease Control and Prevention. NHANES 2003–2004 home page. Available at: http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/nhanes03_04.htm. Accessed September 22, 2008.
17. National Center for Health Statistics. Centers for Disease Control and Prevention. Lab Methods 2003–2004. Available at: http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/lab_methods_03_04.htm. Accessed October 15, 2008.
18. Caldwell KL, Jones RL, Verdon CP, Jarrett JM, Caudill SP, Osterloh JD. Levels of urinary total arsenic and speciated arsenic in the US population: National Health and Nutrition Examination Survey 2003–2004. *J Expo Sci Environ Epidemiol*. 2009;19:59–68.
19. National Center for Health Statistics. Centers for Disease Control and Prevention. Laboratory procedure manual: total arsenic. November, 2007. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/l06uas_c_met_arsenic_total.pdf. Accessed March 10, 2009.
20. National Center for Health Statistics. Centers for Disease Control and Prevention. Laboratory procedure manual: arsenobetaine, arsenocholine, trimethylarsine oxide, monomethylarsonic acid, dimethylarsinic acid, arsenous (II) acid, arsenic (V) Acid. September 2004. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/l06uas_c_met_arsenic_speciated.pdf. Accessed March 10, 2009.
21. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2003;26(suppl 1):S5–S20.
22. Lai VW, Sun Y, Ting E, Cullen WR, Reimer KJ. Arsenic speciation in human urine: are we all the same? *Toxicol Appl Pharm*. 2004;198:297–306.
23. Valdez R, Yoon PW, Liu T, Khoury MJ. Family history and prevalence of diabetes in the US population: the 6-year results from the National Health and Nutrition Examination Survey (1999–2004). *Diabetes Care*. 2007;30:2517–2522.
24. Barr BB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the US population: implications for urinary biologic monitoring measurements. *Environ Health Perspect*. 2005;113:192–200.
25. Biggs ML, Kalman DA, Moore LE, Hopenhayn-Rich C, Smith MT, Smith AH. Relationship of urinary arsenic to intake estimates and a biomarker of effect, bladder cell micronuclei. *Mutat Res*. 1997;386:185–195.
26. Hinwood AL, Sim MR, de Klerk N, Drummer O, Gerostamoulos J, Bastone EB. Are 24-hour urine samples and creatinine adjustment required for analysis of inorganic arsenic in urine in population studies? *Environ Res*. 2002;88:219–224.
27. Nermell B, Lindberg AL, Rahman M, et al. Urinary arsenic concentration adjustment factors and malnutrition. *Environ Res*. 2008;106:212–218.
28. National Center for Health Statistics. Centers for Disease Control and Prevention. Analytic and reporting guidelines. The National Health and Nutrition Examination Survey. Hyattsville, MD: National Center for Health Statistics; 2005:4–8. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf. Accessed September 12, 2008.
29. Rahman M, Tondel M, Ahmad SA, Axelson O. Diabetes mellitus associated with arsenic exposure in Bangladesh. *Am J Epidemiol*. 1998;148:198–203.
30. Lai MS, Hsueh YM, Chen CJ, et al. Ingested inorganic arsenic and prevalence of diabetes mellitus. *Am J Epidemiol*. 1994;139:484–492.
31. Nabi AH, Rahman MM, Islam LN. Evaluation of biochemical changes in chronic arsenic poisoning among Bangladeshi patients. *Int J Environ Res Public Health*. 2005;2:385–393.
32. Rahman M, Tondel M, Chowdhury IA, Axelson O. Relations between exposure to arsenic, skin lesions, and glucosuria. *Occup Environ Med*. 1999;56:277–281.

33. Tsai SM, Wang TN, Ko YC. Mortality for certain diseases in areas with high levels of arsenic in drinking water. *Arch Environ Health*. 1999;54: 186–193.
34. Tseng CH, Tai TY, Chong CK, et al. Long-term arsenic exposure and incidence of non-insulin-dependent diabetes mellitus: a cohort study in arseniasis-hyperendemic villages in Taiwan. *Environ Health Perspect*. 2000;108:847–851.
35. Wang SL, Chiou JM, Chen CJ, et al. Prevalence of non-insulin-dependent diabetes mellitus and related vascular diseases in southwestern arseniasis-endemic and nonendemic areas in Taiwan. *Environ Health Perspect*. 2003;111:155–159.
36. Greenland S. Causation and causal inference. In: Rothman K, Greenland S, eds. *Modern Epidemiology*. 2nd ed. Philadelphia: Lippincott Raven; 1998:7–28.
37. Apostoli P, Bartoli D, Alessio L, Buchet JP. Biological monitoring of occupational exposure to inorganic arsenic. *Occup Environ Med*. 1999; 56:825–832.
38. Hata A, Endo Y, Nakajima Y, et al. HPLC-ICP-MS speciation analysis of arsenic in urine of Japanese subjects without occupational exposure. *J Occup Health*. 2007;49:217–223.
39. Heitland P, Koster HD. Fast determination of arsenic species and total arsenic in urine by HPLC-ICP-MS: concentration ranges for unexposed German inhabitants and clinical case studies. *J Anal Toxicol*. 2008;32: 308–314.
40. Le XC, Cullen WR, Reimer KJ. Human urinary arsenic excretion after one-time ingestion of seaweed, crab, and shrimp. *Clin Chem*. 1994;40: 617–624.

Steinmaus and colleagues have provided a response to the remarks of Navas-Acien et al and the commentary by Longnecker, including further data analysis. Their response is available in the online version of this issue.

—The Editors