PRELIMINARY COMMUNICATION

Arsenic Exposure and Prevalence of Type 2 Diabetes in US Adults

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Context High chronic exposure to inorganic arsenic in drinking water has been related to diabetes development, but the effect of exposure to low to moderate levels of inorganic arsenic on diabetes risk is unknown. In contrast, arsenobetaine, an organic arsenic compound derived from seafood intake, is considered nontoxic.

Objective To investigate the association of arsenic exposure, as measured in urine, with the prevalence of type 2 diabetes in a representative sample of US adults.

Design, Setting, and Participants Cross-sectional study in 788 adults aged 20 years or older who participated in the 2003-2004 National Health and Nutrition Examination Survey (NHANES) and had urine arsenic determinations.

Main Outcome Measure Prevalence of type 2 diabetes across intake of arsenic.

Results The median urine levels of total arsenic, dimethylarsinate, and arsenobetaine were 7.1, 3.0, and 0.9 μg/L, respectively. The prevalence of type 2 diabetes was 7.7%. After adjustment for diabetes risk factors and markers of seafood intake, participants with type 2 diabetes had a 26% higher level of total arsenic (95% confidence interval [CI], 2.0%-56.0%) and a nonsignificant 10% higher level of dimethylarsinate (95% CI, –8.0% to 33.0%) than participants without type 2 diabetes, and levels of arsenobetaine were similar to those of participants without type 2 diabetes. After similar adjustment, the odds ratios for type 2 diabetes comparing participants at the 80th vs the 20th percentiles were 3.58 for the level of total arsenic (95% CI, 1.18-10.83), 1.57 for dimethylarsinate (95% CI, 0.89-2.76), and 0.69 for arsenobetaine (95% CI, 0.33-1.48).

Conclusions After adjustment for biomarkers of seafood intake, total urine arsenic was associated with increased prevalence of type 2 diabetes. This finding supports the hypothesis that low levels of exposure to inorganic arsenic in drinking water, a widespread exposure worldwide, may play a role in diabetes prevalence. Prospective studies in populations exposed to a range of inorganic arsenic levels are needed to establish whether this association is causal.

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INORGANIC ARSENIC IS HIGHLY TOXIC and carcinogenic for humans.[1,2] Millions of individuals worldwide are exposed to drinking water contaminated with inorganic arsenic mainly from natural mineral deposits.[3] In the United States, approximately 13 million individuals live in areas with a concentration of inorganic arsenic in the public water supply that exceeds 10 μg/L, which is the US Environmental Protection Agency's standard for arsenic concentration in public water systems.[4]

Inorganic arsenic at relatively high concentrations increased glucose and insulin levels in animal models,[5] decreased glucose uptake in insulin-sensitive cells,[6-8] and interfered with transcription factors involved in insulin signal transduction and insulin sensitivity in vitro.[8-11] In epidemiologic studies from Taiwan, Bangladesh, and Mexico, high chronic exposure to inorganic arsenic in drinking water (>100 μg/L) was associated with diabetes.[12-18] High chronic exposure to inorganic arsenic in occupational settings was also related to higher levels of glycated hemoglobin, a marker of blood glucose levels.[19] However, the effect of lower levels of exposure to inorganic arsenic on diabetes risk is largely unknown.[20-23]

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In addition to inorganic arsenic, humans are exposed to organic arsenic compounds, such as arsenobetaine and arsenosugars, mainly from seafood.[24] The biotransformation and toxicity of inorganic and organic arsenic compounds differ substantially.[25] Inorganic arsenic compounds (arsenite and arsenate) are metabolized to methylarsonate and dimethylarsinate and excreted in the urine together with unchanged inorganic arsenic.[26] Arsenobetaine, an organic arsenic compound, is excreted unchanged in the urine and is consid-

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ered nontoxic.[27] The relationship of arsenobetaine with diabetes has not been evaluated in human studies.

The 2003-2004 National Health and Nutrition Examination Survey (NHANES) measured for the first time total urine arsenic and urine arsenic species in a representative sample of the US population.[28] Using these data, our objective was to investigate the association of urine arsenic with the prevalence of type 2 diabetes and with the levels of glycated hemoglobin in adult NHANES 2003-2004 participants. Our hypothesis was that exposure to inorganic arsenic is a risk factor for diabetes while exposure to organic arsenic compounds is not.

METHODS

Study Population

NHANES 2003-2004, conducted by the US National Center for Health Statistics, used a complex multistage sampling design to obtain a representative sample of the civilian noninstitutionalized individuals within the US population.[29] The 2003-2004 NHANES study protocols were approved by the institutional review board of the National Center for Health Statistics. Oral and written informed consent was obtained from all participants. The participation rate in NHANES 2003-2004 interviews and physical examinations was 76%. For arsenic measurements, NHANES 2003-2004 randomly selected a one-third random sample of study participants aged 6 years and older (n=2673).[28] Among 1611 participants aged 20 years and older (age threshold was set in accordance with NHANES 2003-2004 questionnaires and questionnaire strategies for adults and to meet our goal of evaluating arsenic and type 2 diabetes in adults), we selected 1027 participants who had fasted 8 to 24 hours before venipuncture. We then excluded 38 pregnant women, 34 participants missing total urine arsenic or urine arsenic species, 24 participants without prior diagnosis of diabetes missing serum glucose, 4 participants missing glycated hemoglobin, 130 participants who reported seafood intake in the past 24 hours, and 9 participants missing other variables of interest, leaving a total of 788 participants for this study.

Urine Arsenic

Spot urine samples (urine samples obtained at the time of the physical examinations) for arsenic analysis were collected in arsenic-free containers, shipped on dry ice, stored frozen at −70°C or lower, and analyzed within 3 weeks of collection.[30] Urine collection and storage materials were screened for arsenic contamination before use.[28] Total arsenic and arsenic species were measured at the Environmental Health Sciences Laboratory of the National Center for Environmental Health following a standardized protocol.[31] Total urine arsenic levels (isotope mass 75) were measured using inductively coupled plasma dynamic reaction cell mass spectrometry on a PerkinElmer ELAN 6100 DRC^{PLUS} or ELAN DRC II ICP-MS (PerkinElmer SCIEX, Concord, ON, Canada).[30] The limit of detection was 0.6 µg/L, and 1.5% of study participants had total urine arsenic levels below the limit of detection. National Institute of Standards and Technology standard reference material 2670 was used for external calibration.[30] The interassay 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 of 8.15 µg/L and 4.07 µg/L, respectively.[31]

Urine arsenic species (arsenite, arsenate, methylarsonate, dimethylarsinate, and arsenobetaine) were measured by inductively coupled plasma dynamic reaction cell mass spectrometry using high-performance liquid chromatography.[32] The limits of detection for arsenite (1.2 µg/L), arsenate (1.0 µg/L), and methylarsonate (0.9 µg/L) were too high for a population exposed to low or moderate arsenic levels, and 96.1%, 93.5%, and 64.8% of sample participants had arsenite, arsenate, and methylarsonate levels below the limit of detection, respectively. As a consequence, arsenite, arsenate, and methylarsonate levels were not used in our analyses. The limits of detection for dimethylarsinate and for arsenobetaine were 1.7 and 0.4 µg/L, respectively. The percent of study participants with levels below the limit of detection were 14.5% for dimethylarsinate and 32.2% for arsenobetaine. The interassay coefficients of variation for quality control–pooled samples with mean dimethylarsinate levels of 6.66 µg/L and mean arsenobetaine levels of 4.87 µg/L were 7% and 10%, respectively.[32] For participants with total arsenic, dimethylarsinate, or arsenobetaine levels below the limit of detection, a level equal to the limit of detection divided by the square root of 2 was imputed.

Diabetes End Points

Serum glucose concentration was measured using a Beckman Synchron LX20 (Beckman Coulter Inc, Fullerton, California).[29] Prevalent type 2 diabetes was defined as a fasting serum glucose level of 126 mg/dL or greater, a self-reported physician diagnosis of diabetes, or self-reported use of insulin or oral hypoglycemic medication. The number of participants with diabetes was 93 (73 with a prior diagnosis). Glycated hemoglobin was measured by a boronate affinity high-performance liquid chromatography system and converted to A_{1C} levels.[29]

Other Variables

Questionnaire information included sex, age, race, and ethnicity; educational, smoking, and alcohol consumption status; and dietary recall interviews for the past 24 hours using an automated multiple pass method.[29] Race and ethnicity were based on self-report, which allowed for multiple options and were subsequently categorized by the National Center for Health Statistics as non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, and other. Seafood intake in the 24 hours immediately preceding the interview was obtained from the 24-hour dietary recall interview results and assigned based on US Department of Agriculture food codes containing fish or seafood as *main dish* or

in combination with other food items.[33] Body mass index was calculated by dividing measured weight in kilograms by measured height in meters squared. Serum cotinine was measured by an isotope-dilution–high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometric method. Blood mercury levels were measured using multielement quadrupole inductively coupled plasma mass spectrometry technology (PerkinElmer Instruments, Shelton, Connecticut). A level equal to the limit of detection divided by the square root of 2 was imputed to those participants with levels below the limit of detection for serum cotinine (18.3% below 0.015 ng/mL [SI conversion: multiply by 5.675 for nmol/L]) or blood mercury (8.0% below 0.2 µg/L [SI conversion: multiply by 4.985 for nmol/L]). Urine creatinine, used to account for urine dilution in spot urine samples, was determined using a Jaffé rate reaction measured with a CX3 analyzer (Beckman Coulter Inc).

Statistical Analysis

All statistical analyses were performed using the survey package in R version 2.4.1 (R Foundation for Statistical Computing, Vienna, Austria) to account for the complex sampling design. Strata, primary sampling units, and special sample weights for arsenic analyses were used to obtain unbiased point estimates and robust linearized standard errors.[28] The statistical significance level was set at $\alpha = .05$. All statistical analyses were 2-sided.

Total arsenic, dimethylarsinate, and arsenobetaine levels were right-skewed and log-transformed for the analyses. For each arsenical, the ratio of geometric mean arsenic concentrations and its 95% confidence interval (CI) in participants with diabetes compared with participants without diabetes were estimated using linear regression models on log-transformed arsenic levels. For risk analyses, logistic regression was used to estimate the odds ratio (OR) of diabetes comparing the 80th vs the 20th percentile of each arsenical distribution and assuming a log-linear dose-response relationship with diabetes. To assess nonlinear relationships, we estimated ORs comparing tertiles 2 and 3 to the lowest tertile of the arsenical distribution, as well as ORs based on restricted quadratic splines with knots at the 5th, 50th, and 95th percentiles.

Although we restricted the analyses to participants who did not report seafood intake in the past 24 hours, we could not remove all the contribution of organic arsenic exposure to total urine arsenic—possibly due to complex metabolism and excretion of organic arsenic compounds, incomplete dietary recall of all seafood sources, and potential presence of arsenobetaine and arsenosugars in unknown sources. We were primarily interested in evaluating the relationship of diabetes with exposure to inorganic arsenic; therefore, our primary assessment of exposure was total urine arsenic concentration adjusted for objective biomarkers of seafood consumption (arsenobetaine and blood mercury). Measures of other organic arsenic compounds derived from seafood, such as arsenosugars, arsenolipids, and their metabolites, are technically challenging and were not available.

Our linear and logistic regression models for total urine arsenic concentrations and diabetes end points were fitted with increasing degrees of adjustment. First, we adjusted for sex, age, race and ethnicity (known determinants of diabetes that may be related to arsenic exposure), and urine creatinine. Second, each model was further adjusted for education, body mass index, serum cotinine, and use of antihypertensive medication. This model represents the association of total arsenic exposure with diabetes independent of the source but adjusted for traditional diabetes risk factors. Third, each model was further adjusted for urine arsenobetaine and blood mercury levels. This model provides estimates for the association of inorganic arsenic not derived from seafood and for arsenobetaine. Further adjustment for smoking status and alcohol intake, as well as exclusion of participants showing levels below the limit of detection, did not modify the observed associations (results not shown). We followed similar analytical strategies to evaluate the relationships of dimethylarsinate and arsenobetaine with type 2 diabetes.

To evaluate the consistency of the findings by participant characteristics, we estimated the ratio of geometric mean total arsenic concentrations by comparing participants with type 2 diabetes with participants without type 2 diabetes for subgroups defined by sex, age, race and ethnicity, education, body mass index, and smoking status.

RESULTS

The median urine levels in the study population were 7.1 µg/L for total arsenic, 3.0 µg/L for dimethylarsinate, and 0.9 µg/L for arsenobetaine (for SI conversion from µg/L to µmol/L, multiply by 0.0133; TABLE 1). After adjusting for sex, age, race, and urine creatinine, urine levels of total arsenic, dimethylarsinate, and arsenobetaine were substantially higher among participants categorized as black, Mexican American, and other race or ethnicity and in participants with high blood mercury levels. Total arsenic was highly correlated with dimethylarsinate (Pearson correlation coefficient for log-transformed variables $r=0.81$) and arsenobetaine ($r=0.76$), and moderately correlated with blood mercury ($r=0.39$). Dimethylarsinate was moderately correlated with arsenobetaine ($r=0.46$) and blood mercury ($r=0.28$). Arsenobetaine and blood mercury were also moderately correlated with one another ($r=0.48$).

In models adjusted for sociodemographic and diabetes risk factors (TABLE 2; models 1 and 2), participants with type 2 diabetes had similar levels of total arsenic and dimethylarsinate, and lower levels of arsenobetaine compared with participants without type 2 diabetes. After adjustment for urine arsenobetaine and blood mercury, biomarkers of seafood intake, participants with type 2 diabetes had 26% higher total arsenic levels (95% CI, 2.0%-56.0%), nonsignificant 10% higher dimethylarsinate levels (95% CI, –8.0% to 33.0%), and similar arseno-

betaine levels than participants without type 2 diabetes (Table 2; model 3).

The ORs for diabetes comparing participants in the 80th vs the 20th percentiles of total urine arsenic were 1.05 (95% CI, 0.57-1.94) before adjustment for biomarkers of seafood intake and 3.58 (95% CI, 1.18-10.83) after adjustment (TABLE 3; models 2 and 3). For urine dimethylarsinate, the corresponding ORs were 1.19 (95% CI, 0.72-1.98) and 1.57 (95% CI, 0.89-2.76), and for urine arsenobetaine they were 0.53 (95% CI, 0.22-1.26) and 0.69 (95% CI, 0.33-1.48). After adjustment for biomarkers of seafood intake, positive associations with increasing total arsenic were also evident in models based on tertiles (Table 3) or on restricted quadratic splines (FIGURE 1).

In sensitivity analyses restricted to 385 participants (50 with type 2 diabetes, 335 without type 2 diabetes) with arsenobetaine levels below the median (≤0.9 µg/L),

Table 1. Urine Arsenic Concentrations by Participant Characteristics

		Urine Arsenic Concentration, Median (IQR), µg/L			*P* Value[b]		
Characteristics	No. (%)[a]	Total Arsenic	Dimethylarsinate	Arsenobetaine	Total Arsenic	Dimethylarsinate	Arsenobetaine
Overall	788	7.1 (3.6-13.9)	3.0 (2.0-5.6)	0.9 (0.3-3.5)			
Sex							
Men	417 (49.4)	8.6 (4.6-14.3)	4.0 (2.3-6.0)	1.1 (0.3-3.8)	.07	.26	.37
Women	371 (50.6)	6.0 (2.9-13.1)	3.0 (2.0-5.0)	0.7 (0.3-3.4)			
Age, y							
20-39	262 (39.3)	7.4 (4.2-15.5)	4.0 (2.0-6.0)	0.8 (0.3-2.9)	.004	.16	.12
40-59	237 (40.3)	6.6 (3.1-11.6)	3.0 (2.0-5.0)	0.9 (0.3-3.5)			
≥60	289 (20.4)	6.8 (3.4-14.5)	3.0 (2.0-5.2)	1.0 (0.3-5.3)			
Race/ethnicity							
White	417 (74.8)	6.2 (3.1-11.6)	3.0 (2.0-5.0)	0.7 (0.3-2.7)	<.001	<.001	.21
Black	172 (11.0)	9.2 (5.4-16.5)	4.0 (2.7-6.3)	1.7 (0.5-5.9)			
Mexican American	160 (8.2)	10.3 (5.9-16.2)	5.1 (3.0-7.0)	1.1 (0.3-3.0)			
Other[c]	39 (6.0)	17.2 (7.2-33.4)	8.0 (4.0-15.3)	2.2 (0.3-12.3)			
Education							
<High school	237 (18.2)	7.4 (3.5-14.8)	3.4 (2.0-6.5)	0.7 (0.3-2.9)	.16	.48	.17
High school	214 (30.2)	7.4 (3.9-15.5)	3.9 (2.0-5.6)	1.1 (0.3-3.7)			
>High school	337 (51.6)	6.9 (3.5-12.7)	3.0 (2.0-5.0)	0.9 (0.3-3.6)			
Body mass index[d]							
<25	245 (32.0)	6.9 (3.3-14.4)	3.0 (2.0-5.9)	0.8 (0.3-3.9)	.05	.07	.23
25-<30	291 (37.9)	7.4 (3.7-13.8)	3.8 (2.0-5.3)	1.1 (0.3-4.3)			
≥30	252 (30.1)	6.4 (4.0-13.9)	3.0 (2.0-5.9)	0.8 (0.3-2.6)			
Smoking							
Never	387 (48.6)	7.0 (3.7-14.4)	3.0 (2.0-5.7)	0.9 (0.3-3.9)	.10	.18	.15
Former	226 (25.7)	7.6 (3.8-14.6)	3.8 (2.0-5.2)	1.4 (0.3-4.5)			
Current	175 (25.8)	6.5 (3.5-11.3)	3.0 (2.0-5.1)	0.7 (0.3-1.7)			
Serum cotinine, ng/mL							
<0.015	144 (14.6)	6.9 (3.2-13.9)	3.1 (2.0-6.0)	0.7 (0.3-3.5)	.22	.14	.60
0.015-<10.0	429 (54.4)	7.3 (4.0-14.8)	3.5 (2.0-5.7)	1.1 (0.3-4.2)			
≥10.0	215 (31.0)	6.7 (3.4-11.6)	3.0 (2.0-5.0)	0.7 (0.3-2.8)			
Arsenobetaine, µg/L							
Tertile 1 (≤0.4)	274 (35.9)	3.4 (1.7-6.5)	2.0 (1.2-4.0)	0.3 (0.3-0.3)	<.001	<.001	
Tertile 2 (>0.4-2.0)	236 (31.1)	6.4 (4.2-9.5)	4.0 (2.0-5.0)	1.0 (0.7-1.4)			
Tertile 3 (>2.0)	278 (33.0)	16.2 (10.5-32.7)	5.0 (3.0-8.0)	7.1 (3.6-15.8)			
Blood mercury, µg/L							
Tertile 1 (<0.6)	271 (33.0)	5.1 (2.9-9.4)	3.0 (2.0-4.3)	0.3 (0.3-0.8)	<.001	.001	<.001
Tertile 2 (0.6-<1.3)	267 (32.3)	7.0 (3.4-12.4)	3.0 (2.0-5.3)	1.0 (0.3-2.6)			
Tertile 3 (≥1.3)	250 (34.7)	10.6 (5.6-21.6)	4.0 (3.0-7.0)	2.5 (0.7-10.4)			
Diabetes							
Yes	93 (7.7)	6.9 (3.0-10.9)	3.0 (2.0-4.3)	0.5 (0.3-2.2)	.60	.73	.03
No	695 (92.3)	7.1 (3.6-14.3)	3.3 (2.0-5.7)	0.9 (0.3-3.6)			

Abbreviation: IQR, interquartile range.
SI conversion factors: To convert total arsenic, dimethylarsinate, or arsenobetaine from µg/L to µmol/L, multiply by 0.0133; for blood mercury from µg/L to nmol/L, multiply by 4.985.
[a] Percentage values are weighted.
[b] *P* values are adjusted for sex, age, race and ethnicity, and urine creatinine level (log-transformed).
[c] Combines *other race* and *other Hispanic* categories due to limited sample size.
[d] Calculated as weight in kilograms divided by height in meters squared.

the ratio of geometric mean total arsenic concentrations in individuals with diabetes vs those without was 1.22 (95% CI, 0.97-1.55) after adjustment for sex, age, race, urine creatinine, education, body mass index, serum cotinine, and hypertension medication. After similar adjustment, the OR of type 2 diabetes was 2.41 (95% CI, 0.69-8.44) comparing participants in the 80th vs 20th percentile of total urine arsenic distribution.

Total urine arsenic and dimethylarsinate levels, but not arsenobetaine, were also positively associated with increasing levels of glycated hemoglobin after adjustment for markers of seafood intake, although the associations were not statistically significant (TABLE 4).

The positive association between total urine arsenic and diabetes after adjustment for markers of seafood intake was consistent for most subgroups examined, with somewhat greater associations in participants who were younger, overweight, and never smokers (FIGURE 2).

COMMENT

In a representative sample of US adults, increasing levels of total urine arsenic were positively associated with type 2 diabetes prevalence and with levels of glycated hemoglobin after adjustment for diabetes risk factors and markers of seafood intake. A nonsignificant association was observed for urine dimethylarsinate and no association was observed for urine arsenobetaine. After adjustment for objective biomarkers of seafood intake, the main source of organic arsenicals, the association of total urine arsenic with diabetes was progressive with no obvious threshold, and consistent for most population subgroups.

These results support our hypothesis that exposure to inorganic arsenic, which in this population was most likely derived from drinking water, is associated with an increased risk of diabetes while exposure to organic arsenicals is not. Our findings extend previous studies conducted in populations exposed to high inorganic arsenic concentrations in drinking water to a population with low- or moderate-arsenic exposure, and suggest that inorganic arsenic may have a role in diabetes development.

Arsenic Exposure

Humans may be exposed to inorganic (arsenite and arsenate) and organic (eg, arsenobetaine, arsenosugars, arsenolip-

Table 2. Ratio of Arsenic Concentrations Comparing Participants With Type 2 Diabetes (n = 93) vs Without (n = 695)

	Total Arsenic	Dimethylarsinate	Arsenobetaine
With diabetes/without diabetes, geometric mean, µg/L	6.2/7.3	3.2/3.5	0.9/1.4
Model 1[a]	0.94 (0.73-1.20)	0.98 (0.84-1.13)	0.62 (0.41-0.96)
Model 2[b]	1.01 (0.80-1.28)	1.02 (0.87-1.20)	0.66 (0.43-1.00)
Model 3[c]	1.26 (1.02-1.56)	1.10 (0.92-1.33)	0.88 (0.63-1.22)

SI conversion factor: To convert total arsenic, dimethylarsinate, or arsenobetaine from µg/L to µmol/L, multiply by 0.0133.
[a] Model 1 is shown as adjusted ratio of geometric mean (95% confidence interval); adjusted for sex, age, race and ethnicity, and urine creatinine level (log-transformed).
[b] Model 2 is shown as adjusted ratio of geometric mean (95% confidence interval); further adjusted for education, body mass index (calculated as weight in kilograms divided by height in meters squared), serum cotinine level (log-transformed), and hypertension medication.
[c] Model 3 is shown as adjusted ratio of geometric mean (95% confidence interval); further adjusted for arsenobetaine (log-transformed) and blood mercury levels (log-transformed), except for arsenobetaine model that was further adjusted for blood mercury only.

Table 3. Odds Ratio of Diabetes by Urine Arsenic Concentrations

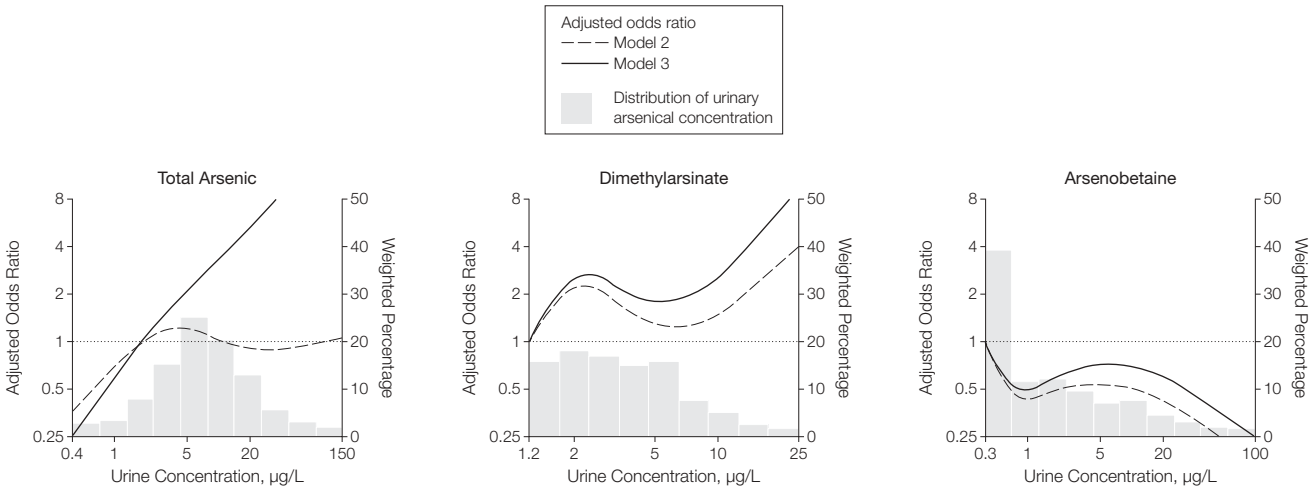
	80th vs 20th Percentile	Tertile 1	Tertile 2	Tertile 3	*P* Value for Trend[a]
Total arsenic, µg/L	16.5 vs 3.0	<4.8	4.8 to 10.8	>10.8	
With diabetes/without diabetes, No.	93/695	29/202	30/230	34/263	
Model 1[b]	0.82 (0.46-1.46)	1 [Reference]	0.89 (0.29-2.79)	0.64 (0.24-1.72)	.48
Model 2[c]	1.05 (0.57-1.94)	1 [Reference]	0.94 (0.25-3.48)	0.74 (0.25-2.20)	.86
Model 3[d]	3.58 (1.18-10.83)	1 [Reference]	1.27 (0.36-4.48)	1.60 (0.46-5.54)	.03
Dimethylarsinate, µg/L	6.0 vs 2.0	<2.4	2.4-5.0	>5.0	
With diabetes/without diabetes, No.	93/695	26/210	42/267	25/218	
Model 1[b]	0.93 (0.54-1.60)	1 [Reference]	0.82 (0.28-2.44)	0.69 (0.28-1.69)	.78
Model 2[c]	1.19 (0.72-1.98)	1 [Reference]	1.01 (0.32-3.23)	0.91 (0.38-2.18)	.47
Model 3[d]	1.57 (0.89-2.76)	1 [Reference]	1.17 (0.43-3.13)	1.22 (0.52-2.86)	.11
Arsenobetaine, µg/L	5.5 vs 0.3	<0.5	0.5-2.0	>2.0	
With diabetes/without diabetes, No.	93/695	40/234	23/213	30/248	
Model 1[b]	0.47 (0.22-1.02)	1 [Reference]	0.51 (0.27-0.93)	0.43 (0.17-1.14)	.05
Model 2[c]	0.53 (0.22-1.26)	1 [Reference]	0.54 (0.28-1.05)	0.44 (0.16-1.21)	.14
Model 3[d]	0.69 (0.33-1.48)	1 [Reference]	0.62 (0.32-1.20)	0.59 (0.23-1.48)	.32

SI conversion factor: To convert total arsenic, dimethylarsinate, or arsenobetaine from µg/L to µmol/L, multiply by 0.0133.
[a] *P* value for trend based on log-transformed arsenic concentrations.
[b] Model 1 is shown as odds ratio (95% confidence interval); adjusted for sex, age, race and ethnicity, and urine creatinine level (log-transformed).
[c] Model 2 is shown as odds ratio (95% confidence interval); further adjusted for education, body mass index (calculated as weight in kilograms divided by height in meters squared), serum cotinine level (log-transformed), and hypertension medication.
[d] Model 3 is shown as odds ratio (95% confidence interval); further adjusted for arsenobetaine (log-transformed) and blood mercury levels (log-transformed), except for arsenobetaine models that were further adjusted for blood mercury only.

ids) arsenic compounds.[24,25] The main sources of inorganic arsenic are contaminated drinking water and food.[3,34-36] In the United States, contaminated water from naturally occurring inorganic arsenic in rocks and soils is common in certain areas of the West, Midwest, and Northeast regions. It is estimated that 8% of public water supply systems in the United States may exceed arsenic levels of 10 µg/L, 14% may exceed levels of 5 µg/L, and 25% may exceed levels of 2 µg/L.[35] The US Environmental Protection Agency's standard for arsenic concentrations in drinking water (10 µg/L) was based on quantitative estimates of the effect of arsenic on cancer incidence.[2] Foods such as flour and rice can also provide small quantities of inorganic arsenic, particularly if grown or cooked in areas with arsenic contamination in soil and water.[37] Estimated daily dietary intake of inorganic arsenic in the United States ranges from 8.4 to 14 µg/d for various age groups.[36]

Seafood is the main source of organic arsenic compounds in the human diet.[24] Most fish and shellfish are rich in arsenobetaine that is rapidly excreted unchanged in urine. Based on toxicity ex-

Figure 1. Odds Ratio of Diabetes by Urine Arsenic Concentrations



Lines represent adjusted odds ratios based on restricted quadratic splines for log-transformed arsenic concentrations with knots at 5th, 50th, and 95th percentiles. The reference value was set at the 10th percentile of each arsenical distribution. Odds ratios were adjusted for sex, age, race and ethnicity, urine creatinine level, education, body mass index (calculated as weight in kilograms divided by height in meters squared), serum cotinine and hypertension medication for model 2, and further adjusted for arsenobetaine (except arsenobetaine model) and blood mercury for model 3. Bars represent the weighted histogram of each arsenical distribution. For urinary arsenical distributions, 10 equally sized bins were selected from the 1st to the 99th percentiles of each log-transformed arsenical distribution except for dimethylarsinate. For dimethylarsinate, 9 equally sized bins were selected to improve the shape of the histogram (many dimethylarsinate measurements were whole numbers).

Table 4. Mean Difference in Percent Levels of Glycated Hemoglobin by Urine Arsenic Concentrations

	80th vs 20th Percentile	Tertile 1	Tertile 2	Tertile 3	*P* Value for Trend[a]
Total arsenic, µg/L	16.5 vs 3.0	<4.8	4.8 to 10.8	>10.8	
Model 1[b]	–0.04 (–0.16 to 0.08)	0 [Reference]	–0.03 (–0.16 to 0.09)	0.00 (–0.20 to 0.19)	.52
Model 2[c]	0.00 (–0.13 to 0.13)	0 [Reference]	0.01 (–0.12 to 0.13)	0.05 (–0.16 to 0.26)	.95
Model 3[d]	0.18 (–0.20 to 0.56)	0 [Reference]	0.08 (–0.07 to 0.23)	0.23 (–0.18 to 0.63)	.32
Dimethylarsinate, µg/L	6.0 vs 2.0	<2.4	2.4 to 5.0	>5.0	
Model 1[b]	0.01 (–0.16 to 0.18)	0 [Reference]	–0.07 (–0.18 to 0.03)	–0.04 (–0.26 to 0.18)	.88
Model 2[c]	0.05 (–0.13 to 0.23)	0 [Reference]	–0.02 (–0.14 to 0.10)	0.01 (–0.23 to 0.25)	.59
Model 3[d]	0.11 (–0.12 to 0.33)	0 [Reference]	0.02 (–0.11 to 0.14)	0.09 (–0.20 to 0.38)	.33
Arsenobetaine, µg/L	5.5 vs 0.3	<0.5	0.5 to 2.0	>2.0	
Model 1[b]	–0.10 (–0.23 to 0.03)	0 [Reference]	–0.13 (–0.33 to 0.06)	–0.11 (–0.31 to 0.08)	.13
Model 2[c]	–0.07 (–0.18 to 0.04)	0 [Reference]	–0.15 (–0.32 to 0.02)	–0.11 (–0.29 to 0.07)	.20
Model 3[d]	0.01 (–0.11 to 0.13)	0 [Reference]	–0.11 (–0.26 to 0.03)	–0.01 (–0.18 to 0.17)	.87

SI conversion factor: To convert total arsenic, dimethylarsinate, or arsenobetaine from µg/L to µmol/L, multiply by 0.0133.
[a] *P* value for trend based on log-transformed arsenic concentrations.
[b] Model 1 is shown as mean difference (95% confidence interval); adjusted for sex, age, race and ethnicity, and urine creatinine level (log-transformed).
[c] Model 2 is shown as mean difference (95% confidence interval); further adjusted for education, body mass index (calculated as weight in kilograms divided by height in meters squared), serum cotinine level (log-transformed), and hypertension medication.
[d] Model 3 is shown as mean difference (95% confidence interval); further adjusted for arsenobetaine (log-transformed) and blood mercury levels (log-transformed), except for arsenobetaine models that were further adjusted for blood mercury only.

periments, arsenobetaine is considered nontoxic.[27] In our study, urine arsenobetaine was not related to diabetes or glycated hemoglobin after adjustment for blood mercury. Seaweed and some seafood such as scallops and mussels are also rich in arsenosugars that are metabolized to several compounds (including dimethylarsinate) that contribute to total urine arsenic levels.[38,39] In our study, we adjusted the relationship of total arsenic and dimethylarsinate with diabetes for objective markers of seafood intake and thus, we indirectly controlled for arsenosugars. Although we were unable to evaluate the relationship of exposure to arsenosugars with diabetes, toxicity experiments seem to indicate that arsenosugars and their metabolites are either nontoxic or have very low toxicity compared with inorganic arsenic.[38]

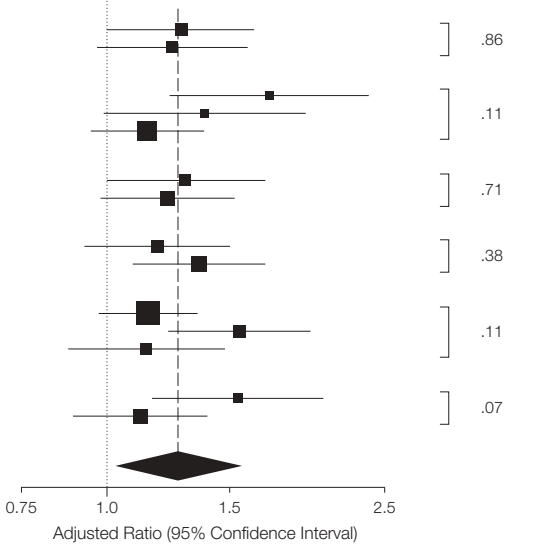
Inorganic Arsenic and Diabetes

High levels of inorganic arsenic in drinking water have been associated with increased risk of type 2 diabetes in Taiwan, Bangladesh, and Mexico.[12-14,18] In a meta-analysis comparing extreme arsenic exposure categories (village drinking water levels or living in a high- vs low-arsenic area) in studies from Taiwan and Bangladesh, the pooled relative risk for diabetes was 2.52 (95% CI, 1.69-3.75).[16] In these studies, arsenic exposure was determined based on ecologic measures of arsenic in drinking water, but no information on arsenic exposure was available at the individual level. In a case-control study in Mexico, the OR for diabetes comparing the highest tertile (>104 μg/g creatinine) to the lowest tertile (<64 μg/g creatinine) of total urine arsenic concentrations was 2.84 (95% CI, 1.64-4.92).[15] Since these studies were conducted in populations exposed to high arsenic levels and arsenic exposure was mostly assessed ecologically, the implications for diabetes in populations exposed to lower levels have been debated.[16,40,41]

Few studies have addressed the association of low or moderate exposure to inorganic arsenic with diabetes risk.[20-22] In 660 participants exposed to relatively low arsenic levels in drinking water in Central Taiwan, hair arsenic levels were associated with elevated plasma glucose levels and with the prevalence of the metabolic syndrome.[23] In Utah, an ecologic study found no association between arsenic levels in drinking water and diabetes mortality after controlling only for age and sex.[21] In a cross-sectional study in Wisconsin, the age, sex, body mass index, and smoking status adjusted OR for self-reported diabetes comparing participants with arsenic levels in drinking water between 2 and 10 μg/L and levels greater than 10 μg/L to participants with arsenic levels in drinking water of less than 2 μg/L were 1.35 (95% CI, 0.78-2.33) and 1.02 (95% CI, 0.49-2.15), respectively.[20] Biomarkers of arsenic exposure were not measured in this study. In a small case-control study in southern Spain, 38 participants with diabetes had similar total urine arsenic concentrations (mean level 3.44 μg/L) compared with 49 control participants (mean level 3.68 μg/L), but the study was not adjusted for diabetes

Figure 2. Urine Arsenic Concentrations Comparing Participants With Type 2 Diabetes vs Participants Without

Participant Subgroup	Total Urine Arsenic Concentration, Geometric Mean, μg/L: Participants With Type 2 Diabetes	Total Urine Arsenic Concentration, Geometric Mean, μg/L: Participants Without Type 2 Diabetes	Adjusted Ratio (95% Confidence Interval)[a]	*P* for Interaction
Sex				
Men	8.2	8.4	1.28 (1.00-1.63)	.86
Women	4.3	6.5	1.24 (0.97-1.59)	
Age, y				
20-39	10.8	8.2	1.71 (1.23-2.37)	.11
40-59	4.3	6.7	1.38 (0.99-1.93)	
≥60	8.1	6.9	1.14 (0.94-1.38)	
Race/ethnicity				
White	5.1	6.3	1.30 (1.00-1.69)	.71
Other	11.6	11.4	1.22 (0.98-1.52)	
Education				
≤High school	6.1	8.1	1.18 (0.92-1.50)	.38
>High school	6.4	6.7	1.35 (1.09-1.68)	
Body mass index[b]				
<25	5.9	7.3	1.14 (0.97-1.35)	.11
25-<30	9.6	7.5	1.55 (1.23-1.96)	
≥30	5.4	7.1	1.14 (0.88-1.48)	
Smoking				
Never	5.6	7.4	1.54 (1.16-2.04)	.07
Ever	6.7	7.3	1.12 (0.89-1.39)	
Overall	6.2	7.3	1.26 (1.02-1.56)	



[a]Ratio (95% confidence interval) is also adjusted for log-transformed serum cotinine, hypertension medication, arsenobetaine, and blood mercury.
[b]Body mass index is calculated as weight in kilograms divided by height in meters squared.
The area of each data marker is proportional to the inverse of the variance of the adjusted ratio.

risk factors or for markers of seafood intake.[22]

The potential role of arsenic in diabetes development is supported by experimental and mechanistic evidence. Rats administered 1.7 mg/kg of sodium arsenite by gavage for 90 days had higher glucose and insulin levels, lower glucose-to-insulin ratio, and higher homeostasis model assessment of insulin resistance compared with control participants.[5] Insulin synthesis and secretion was impaired in pancreatic β cells treated with arsenite 0.5 to 10 µmol for 72 to 144 hours.[42] In insulin-sensitive cells exposed both to insulin and arsenite, glucose uptake decreased compared with insulin alone.[6-8] Glucose uptake was also inhibited in cells exposed to methylarsonite for 4-hour or 24-hour periods.[6,8] Other potential mechanisms include arsenic influence on the expression of gene transcription factors related to insulin signal transduction,[9,43,44] adipocyte differentiation, and insulin sensitivity.[8,10,11] Finally, arsenic could induce diabetes by nonspecific mechanisms such as oxidative stress, inflammation, or apoptosis, mechanisms that have been related both to arsenic exposure and diabetes development. However, most mechanistic experiments have been conducted at high-arsenic concentrations and further research is needed to establish the pathways potentially affected by arsenic at low and moderate levels of exposure. Experimental evidence will also be essential to investigate important biological questions on the role of arsenic in diabetes, such as the arsenic effect and metabolism at the cellular level.[6,8,45]

Strengths and Limitations

Important strengths of this study include the use of a representative sample of the general US population; the use of urine arsenic, the biomarker recommended by the US National Research Council Subcommittee on Arsenic in Drinking Water,[2] to assess exposure; the adjustment for relevant diabetes risk factors and for biomarkers of seafood intake; and the rigorous quality control of study procedures in NHANES.

While the public health and research implications of this study are important, some limitations must also be considered. First, our analysis was limited by a relatively small sample size because NHANES 2003-2004 measured arsenic in just a random third of survey participants. Second, the study is cross-sectional and temporality between urine arsenic levels and diabetes development cannot be completely ensured. It is unknown if diabetes alters the excretion and metabolism of arsenic. Prospective epidemiologic studies in populations exposed to a wide range of inorganic arsenic are needed to confirm this association. Third, because of its relatively short half-life, urine arsenic may not reflect long-term exposure. In the absence of public health interventions, however, arsenic concentrations in drinking water are relatively stable over time,[46-48] resulting in steady urine arsenic levels. Indeed, repeated urine samples in populations with low-seafood intake have shown relatively constant urine arsenic levels over time reflecting ongoing chronic exposure to inorganic arsenic from drinking water.[49] Fourth, the high limits of detection for arsenite, arsenate, and methylarsonate (species that more readily reflect exposure to inorganic arsenic), precluded the use of those species in the analyses. For total arsenic, dimethylarsinate, and arsenobetaine, the relatively high coefficients of variation, limits of detection, or both could have resulted in substantial misclassification and potential underestimation of the associations. Other limitations include the use of spot urine samples and the need to adjust for urine creatinine levels to account for urine dilution and the possibility of residual confounding by geographical location or by urbanization.

CONCLUSIONS

We found a positive association between total urine arsenic, likely reflecting inorganic arsenic exposure from drinking water and food, with the prevalence of type 2 diabetes in a population with low to moderate arsenic exposure. Together with the experimental and epidemiologic evidence supporting a diabetes effect for high levels of arsenic exposure, these findings reinforce the need to evaluate the role of inorganic arsenic in diabetes development in high-quality prospective studies conducted in populations exposed to a wide range of arsenic levels.

From a public health perspective, confirmation of a role for arsenic in diabetes development would add to the concerns posed by the carcinogenic, cardiovascular, developmental, and reproductive effects of inorganic arsenic in drinking water,[1,2,50,51] and could substantially modify risk assessment and risk-benefit analyses estimating the consequences of arsenic exposure. Given widespread exposure to inorganic arsenic from drinking water worldwide, elucidating the contribution of arsenic to the diabetes epidemic is a public health research priority with potential implications for the prevention and control of diabetes.

Author Contributions: Dr Navas-Acien had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Navas-Acien, Silbergeld, Guallar.
Acquisition of data: Navas-Acien.
Analysis and interpretation of data: Navas-Acien, Silbergeld, Pastor-Barriuso, Guallar.
Drafting of the manuscript: Navas-Acien, Silbergeld, Pastor-Barriuso, Guallar.
Critical revision of the manuscript for important intellectual content: Navas-Acien, Silbergeld, Pastor-Barriuso, Guallar.
Statistical analysis: Navas-Acien, Pastor-Barriuso, Guallar.
Administrative, technical, or material support: Navas-Acien.
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