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# Association Between Exposure to Low to Moderate Arsenic Levels and Incident Cardiovascular Disease

## A Prospective Cohort Study

Katherine A. Moon, MPH; Eliseo Guallar, MD, DrPH; Jason G. Umans, MD, PhD; Richard B. Devereux, MD; Lyle G. Best, MD; Kevin A. Francesconi, PhD; Walter Goessler, PhD; Jonathan Pollak, MPP; Ellen K. Silbergeld, PhD; Barbara V. Howard, PhD; and Ana Navas-Acien, MD, PhD

**Background:** Long-term exposure to high levels of arsenic is associated with increased risk for cardiovascular disease, whereas risk from long-term exposure to low to moderate arsenic levels (<100 μg/L in drinking water) is unclear.

**Objective:** To evaluate the association between long-term exposure to low to moderate arsenic levels and incident cardiovascular disease.

**Design:** Prospective cohort study.

**Setting:** The Strong Heart Study baseline visit between 1989 and 1991, with follow-up through 2008.

**Patients:** 3575 American Indian men and women aged 45 to 74 years living in Arizona, Oklahoma, and North and South Dakota.

**Measurements:** The sum of inorganic and methylated arsenic species in urine at baseline was used as a biomarker of long-term arsenic exposure. Outcomes were incident fatal and nonfatal cardiovascular disease.

**Results:** A total of 1184 participants developed fatal and nonfatal cardiovascular disease. When the highest and lowest quartiles of arsenic concentrations (>15.7 vs. <5.8 μg/g creatinine) were compared, the hazard ratios for cardiovascular disease, coronary heart disease, and stroke mortality after adjustment for sociodemographic factors, smoking, body mass index, and lipid levels were 1.65 (95% CI, 1.20 to 2.27; *P* for trend < 0.001), 1.71 (CI, 1.19 to 2.44; *P* for trend < 0.001), and 3.03 (CI, 1.08 to 8.50; *P* for trend = 0.061), respectively. The corresponding hazard ratios for incident cardiovascular disease, coronary heart disease, and stroke were 1.32 (CI, 1.09 to 1.59; *P* for trend = 0.002), 1.30 (CI, 1.04 to 1.62; *P* for trend = 0.006), and 1.47 (CI, 0.97 to 2.21; *P* for trend = 0.032). These associations varied by study region and were attenuated after further adjustment for diabetes, hypertension, and kidney disease measures.

**Limitation:** Direct measurement of individual arsenic levels in drinking water was unavailable.

**Conclusion:** Long-term exposure to low to moderate arsenic levels was associated with cardiovascular disease incidence and mortality.

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For author affiliations, see end of text.

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Inorganic arsenic in water and food (particularly rice and grain) is a major global health problem (1). High arsenic levels in drinking water (>100 μg/L) increased the risk for peripheral artery disease, coronary heart disease, stroke, and carotid atherosclerosis in studies conducted in Taiwan (2–4), Bangladesh (5, 6), Chile (7), Inner Mongolia (8, 9), and Pakistan (10, 11). Less is known about the cardiovascular effects of low to moderate arsenic levels (<100 μg/L in drinking water) in most populations because of a lack of prospective studies, limitations in exposure and outcome assessment, and inadequate information on cardiovascular risk factors (12, 13). Indeed, the risk–benefit analysis that established the current U.S. standard for arsenic in drinking water (10 μg/L) did not quantify the effect of arsenic on cardiovascular disease because of a lack of adequate data (14).

In the United States, exposure to arsenic in drinking water disproportionately affects small rural communities in the Southwest, Midwest, and Northeast (15). The Strong Heart Study is a population-based prospective cohort study of cardiovascular disease among 3 American Indian communities in rural Arizona, Oklahoma, and North and South Dakota ("the Dakotas") (16). At the time of the study, arsenic levels in public drinking water systems were less than 10 μg/L to 61 μg/L in Arizona, less than 10 μg/L in Oklahoma, and less than 1 μg/L to 21 μg/L in the Dakotas (17). In private wells, arsenic levels probably exceeded 10 μg/L (and possibly 50 μg/L in Arizona and the Dakotas) (15). In Arizona and the Dakotas, drinking water was probably the main source of inorganic arsenic in study participants. Among participants in Oklahoma, as in other populations with low arsenic levels in drinking water (18, 19), diet was probably the main source of arsenic exposure. Potential dietary sources of inorganic arsenic in the Strong Heart Study communities include rice, flour, and other grains (tacos, fry bread, and tortillas are especially common grain-based items). Thus, the objective of this study was to examine the prospective association of long-term arsenic

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***Context***

Long-term exposure to high levels of inorganic arsenic in water and food, particularly rice and grains, is associated with increased risk for cardiovascular disease.

***Contribution***

This prospective cohort study involving 3575 rural American Indian men and women found that long-term exposure to low to moderate levels of arsenic, as measured in urine, was associated with increased risk for fatal and nonfatal cardiovascular disease.

***Caution***

Inorganic arsenic in the water or food that participants consumed was not directly measured. Causality was not clearly established.

***Implication***

Exposure to low to moderate levels of inorganic arsenic may be associated with risk for cardiovascular disease.

*—The Editors*

exposure with cardiovascular disease over almost 20 years of follow-up in the Strong Heart Study.

## METHODS

### Study Population

The Strong Heart Study examined 4549 men and women aged 45 to 74 years at baseline between 1989 and 1991. Participants were invited to subsequent clinical visits between 1993 and 1995 and between 1998 and 1999 and were actively followed through 2008 (**Appendix Figure 1**, available at www.annals.org). Every eligible person was invited to participate in Arizona and Oklahoma, whereas a cluster sampling technique was used in the Dakotas (20). The participation rate was 62% (21). Most participants were born in their communities and had lived there for their entire lives. Compared with nonparticipants, participants were similar in age, body mass index, and self-reported frequency of diabetes but were more likely to be female and to have self-reported hypertension (21). The Indian Health Service, institutional review boards, and participating tribes approved the study protocol. All participants provided informed consent.

We used data from 3973 participants with sufficient urine available for arsenic measurements. We then excluded 273 participants with self-reported or clinical cardiovascular disease at baseline, 3 who were missing urinary creatinine data, and 122 who were missing other variables of interest, leaving 3575 participants for this analysis. Included participants were similar to those who were excluded because of missing data (not shown).

### Data Collection

The clinical examinations consisted of a personal interview, physical examination, fasting blood test, and spot urine sample collection (20). Trained and certified interviewers administered standardized questionnaires, and centrally trained nurses and medical assistants measured height, weight, and systolic and diastolic blood pressures and collected blood and urine according to standardized protocols (20). Methods to measure blood pressure, cholesterol level, fasting glucose level, oral glucose tolerance, hemoglobin $A_{1c}$ level, and plasma creatinine level have been described (20). We defined hypertension as systolic blood pressure of 140 mm Hg or greater, diastolic blood pressure of 90 mm Hg or greater, or antihypertensive medication use. Low-density lipoprotein (LDL) cholesterol levels were calculated using the Friedewald equation for participants with triglyceride levels less than 4.5 mmol/L (<400 mg/dL), with missing values replaced by LDL levels measured using the $\beta$ quantification method. We defined albuminuria as a urinary albumin–creatinine ratio of 30 mg/g or greater (22). We used the Chronic Kidney Disease Epidemiology Collaboration formula (23) to calculate estimated glomerular filtration rate (eGFR) from recalibrated plasma creatinine measurements, age, and sex. Diabetes was defined as a fasting glucose level 7.0 mmol/L or greater (≥126 mg/dL), 2-hour postload plasma glucose level 11.1 mmol/L or greater (≥200 mg/dL), hemoglobin $A_{1c}$ level 6.5% or greater, or self-reported use of insulin or an oral hypoglycemic agent.

Spot urine samples collected the morning of the baseline clinical examination were frozen within 1 to 2 hours of collection and stored at −70 °C or lower (20). Urinary albumin and creatinine levels were measured by an automated nephelometric immunochemical procedure and an automated alkaline picrate method, respectively (20).

### Urinary Arsenic

To assess long-term arsenic exposure, we measured arsenic species concentrations in urine. The analytic methods and associated quality control criteria for arsenic analysis have been described in detail (24). Arsenic species concentrations were determined by high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry that served as the arsenic selective detector (Agilent 1100 HPLC and Agilent 7700x ICP-MS, Agilent Technologies, Santa Clara, California). Arsenic speciation distinguishes species directly related to inorganic arsenic exposure (arsenite, arsenate, monomethylarsonate [MMA], and dimethylarsinate [DMA]) from those related to organic arsenicals in seafood (arsenobetaine as an overall marker of seafood arsenicals), which are generally considered nontoxic (1). The limit of detection for inorganic arsenic (arsenite plus arsenate), MMA, DMA, and arsenobetaine plus other cationic arsenic species was 0.1 $\mu$g/L (24). For participants with arsenic species concentrations below the limit of detection (5.1% for inorganic arsenic, 0.8% for MMA, 0.03% for DMA, and 2.1% for arsenobetaine), levels were imputed as the limit of detection divided by the square root of 2. The interassay coefficients of

variation for inorganic arsenic, MMA, DMA, and arsenobetaine for an in-house reference urine sample were 6.0%, 6.5%, 5.9%, and 6.5%, respectively (24).

We used the sum of urinary inorganic arsenic (arsenite and arsenate) and methylated arsenic species (DMA and MMA) as a biomarker to integrate inorganic arsenic exposure from multiple sources (1, 25, 26). To account for urine dilution, urinary arsenic concentrations were divided by urinary creatinine levels and expressed as "μg/g creatinine." Low urinary concentrations of arsenobetaine (median, 0.76 μg/g creatinine; interquartile range, 0.48 to 1.70 μg/g creatinine) confirmed that seafood intake was low in this sample, indicating that measured methylated species reflect inorganic arsenic exposure. Inorganic arsenic, MMA, and DMA have estimated half-lives of 38, 9, and 2 days, respectively (27, 28). In a random sample stratified by study region of 380 participants with 3 repeated arsenic measures over 10 years, the intraclass correlation coefficient for the log-transformed sum of inorganic and methylated arsenic species was 0.64 (95% CI, 0.60 to 0.69) and the average change in urinary arsenic concentrations between the first and third visits was −0.8 μg/g creatinine.

### Cardiovascular Disease Incidence and Mortality Follow-up

Incident cardiovascular end points during follow-up were identified by annual contact, by review of hospitalization and death records, and during 2 clinic visits conducted between 1993 and 1995 and between 1998 and 1999. Follow-up through 2008 was 99.8% complete for mortality and 99.2% complete for nonfatal events. When possible cardiovascular events were identified, medical records were abstracted and mortality and morbidity review committees adjudicated cardiovascular events (20). Detailed definitions of the criteria used by the review committees have been described previously (20, 29) and are included in the **Supplement** (available at www.annals.org).

We defined incident coronary heart disease as the first occurrence of definite nonfatal coronary heart disease or definite and possible fatal coronary heart disease. Incident stroke was defined as the first occurrence of a definite nonfatal stroke or a definite or possible fatal stroke. We defined incident cardiovascular disease as the first occurrence of coronary heart disease or stroke, as previously defined; definite nonfatal congestive heart failure; or other fatal cardiovascular disease.

Follow-up extended from the date of the baseline examination until the date of the cardiovascular event, the date of death, or 31 December 2008, whichever occurred first. The mean follow-up time among participants without a cardiovascular event was 15.0 years.

### Statistical Analysis

We evaluated the prospective association of urinary arsenic concentrations with incident cardiovascular disease by using Cox proportional hazards models with age as time scale and individual entry times (age at baseline) treated as staggered entries. Urinary arsenic concentrations were modeled as quartiles, log-transformed concentrations to compare the 75th and 25th percentiles (interquartile range), and log-transformed concentrations with restricted quadratic splines. We allowed the nonparametric underlying baseline hazards to differ by region because study locations differed by urinary arsenic concentrations (17) and cardiovascular risk factors (16). Models were progressively adjusted (see footnotes of **Tables 1** and **2**). *P* values for linear and nonlinear trends were obtained from Wald tests for log-transformed arsenic coefficients and restricted quadratic spline coefficients, respectively. We found no violations of the proportional hazards assumption over time based on visual examinations of smoothed association between age and scaled Schoenfeld residuals over time and a test for a nonzero slope for this association (30). To estimate absolute rates, we used Poisson regression to model cardiovascular disease incidence and mortality and then estimated the marginal response for each arsenic quartile given mean values of covariates. Poisson models were adjusted for model 2 covariates, age, and study region.

We conducted several sensitivity analyses. First, we evaluated the association of cardiovascular disease end points with arsenobetaine, a nontoxic seafood arsenical (1). We hypothesized that arsenobetaine would not be associated with cardiovascular disease. Second, we evaluated alternative methods to adjust spot urine samples for urine dilution. We measured urinary arsenic concentrations in μg/L and adjusted the models for log-transformed urinary creatinine concentrations. For participants without diabetes or albuminuria ($n = 1646$), we also used urinary arsenic concentrations adjusted to the mean specific gravity of 1.017 (31). The specific gravity of urine is less dependent on muscle mass and nutritional status than urinary creatinine (31), but this adjustment is inadequate if albumin or glucose is present in urine (32). Both analyses resulted in similar findings (not shown).

We performed subgroup analyses to evaluate effect modification in adjusted models by including quantitative interaction terms for log-transformed urinary arsenic concentrations with indicator variables for age groups; sex; smoking status; diabetes status; study region; and proportions of inorganic arsenic, MMA, and DMA in separate models. On the basis of prior evidence (6), we hypothesized that the association between arsenic exposure and incident cardiovascular disease would be stronger in current smokers and in participants with higher proportions of MMA and lower proportions of DMA in urine. Other subgroup analyses were exploratory without a priori hypotheses. *P* values for interactions were obtained using Wald tests for multiple coefficients.

Arsenic methylation patterns have been related to differences in cardiovascular end points in populations from Taiwan (4, 33). To evaluate the potential role of arsenic metabolism, we examined the relationship between the

*Table 1.* **Cardiovascular Mortality End Points, by Urinary Arsenic Concentration* ($n$ = 3575)**

| Variable | Quartile of Inorganic Plus Methylated Arsenic Species ($\mu$g/g creatinine) | | | | 75th vs. 25th Percentile‡ | *P* Value for Trend§ |
|---|---|---|---|---|---|---|
| | <5.8 (4.2)† | 5.8–9.7 (7.5)† | 9.8–15.7 (12.4)† | >15.7 (21.8)† | | |
| **Cardiovascular disease mortality** | | | | | | |
| Cases, *n* | 86 | 95 | 115 | 143 | 439 | – |
| Person-years | 13 616 | 13 430 | 12 720 | 12 033 | 51 799 | – |
| Hazard ratio (95% CI)‖ | | | | | | |
| Model 1¶ | 1 (referent) | 1.06 (0.78–1.43) | 1.21 (0.89–1.65) | 1.59 (1.17–2.17) | 1.34 (1.15–1.56) | <0.001 |
| Model 2** | 1 (referent) | 1.12 (0.83–1.52) | 1.26 (0.92–1.73) | 1.65 (1.20–2.27) | 1.36 (1.16–1.58) | <0.001 |
| Model 3†† | 1 (referent) | 1.06 (0.78–1.44) | 1.24 (0.90–1.70) | 1.52 (1.10–2.11) | 1.35 (1.15–1.58) | <0.001 |
| Model 4‡‡ | 1 (referent) | 1.02 (0.75–1.39) | 1.15 (0.84–1.58) | 1.29 (0.93–1.79) | 1.25 (1.06–1.47) | 0.007 |
| **Coronary heart disease mortality** | | | | | | |
| Cases, *n* | 68 | 67 | 87 | 119 | 341 | – |
| Person-years | 13 616 | 13 430 | 12 720 | 12 033 | 51 799 | – |
| Hazard ratio (95% CI)‖ | | | | | | |
| Model 1¶ | 1 (referent) | 0.91 (0.65–1.30) | 1.11 (0.78–1.57) | 1.59 (1.13–2.25) | 1.37 (1.15–1.63) | <0.001 |
| Model 2** | 1 (referent) | 0.99 (0.70–1.41) | 1.18 (0.83–1.69) | 1.71 (1.19–2.44) | 1.41 (1.18–1.68) | <0.001 |
| Model 3†† | 1 (referent) | 0.93 (0.65–1.32) | 1.15 (0.80–1.66) | 1.57 (1.08–2.27) | 1.40 (1.17–1.68) | <0.001 |
| Model 4‡‡ | 1 (referent) | 0.89 (0.62–1.27) | 1.06 (0.74–1.53) | 1.33 (0.92–1.93) | 1.30 (1.08–1.57) | 0.005 |
| **Stroke mortality** | | | | | | |
| Cases, *n* | 6 | 17 | 13 | 18 | 54 | – |
| Person-years | 13 616 | 13 430 | 12 720 | 12 033 | 51 799 | – |
| Hazard ratio (95% CI)‖ | | | | | | |
| Model 1¶ | 1 (referent) | 1.37 (0.53–3.55) | 2.46 (0.88–6.83) | 3.66 (1.34–10.03) | 1.66 (1.11–2.48) | 0.014 |
| Model 2** | 1 (referent) | 1.41 (0.54–3.67) | 2.16 (0.77–6.09) | 3.03 (1.08–8.50) | 1.51 (0.98–2.32) | 0.061 |
| Model 3†† | 1 (referent) | 1.40 (0.54–3.65) | 2.05 (0.72–5.81) | 2.75 (0.97–7.81) | 1.48 (0.95–2.32) | 0.082 |
| Model 4‡‡ | 1 (referent) | 1.30 (0.50–3.39) | 1.97 (0.70–5.55) | 2.35 (0.83–6.69) | 1.37 (0.87–2.14) | 0.175 |

* Sum of inorganic and methylated arsenic species (dimethylarsinate and monomethylarsonate).
† Range (median).
‡ Comparison of the 75th and 25th percentiles (interquartile range) of the sum of inorganic and methylated urinary arsenic concentrations (15.7 vs. 5.8 $\mu$g/g creatinine).
§ Obtained from Cox proportional hazards models with log-transformed arsenic as a continuous variable.
‖ Adjustment for systolic blood pressure and hypertension medication use instead of hypertension and for hemoglobin $A_{1c}$ levels instead of diabetes produced similar results (data not shown).
¶ Stratified by study center and adjusted for age (age as time metric and age at baseline were treated as staggered entries).
** Further adjusted for sex, education (none, some, or high school), smoking status (never, former, or current), body mass index (kg/m$^2$), and low-density lipoprotein cholesterol level (mg/dL).
†† Further adjusted for hypertension (yes or no), diabetes (yes or no), and estimated glomerular filtration rate (mL/min/1.73 m$^2$).
‡‡ Further adjusted for albuminuria (yes or no).

relative proportions of arsenic species in urine (log-transformed proportions of inorganic arsenic, MMA, and DMA) and incident cardiovascular disease on the subset of participants with detectable inorganic arsenic, MMA, and DMA ($n$ = 3381).

Statistical analyses were performed with R, version 2.5.1 (R Foundation for Statistical Computing, Vienna, Austria [www.r-project.org]), and Stata/IC, version 12 (StataCorp, College Station, Texas).

### Role of the Funding Source

The funders played no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript.

## RESULTS

Over 45 738 person-years of follow-up, 439 participants died of cardiovascular disease (341 coronary heart disease deaths and 54 stroke deaths) and 1184 participants developed fatal or nonfatal cardiovascular disease (846 incident coronary heart disease events and 264 incident strokes). Overall, the median urinary arsenic concentration was 9.7 $\mu$g/g creatinine (interquartile range, 5.8 to 15.7 $\mu$g/g creatinine; range, 0.1 to 183.4 $\mu$g/g creatinine). Urinary arsenic concentrations varied by study region; the medians were 14.2, 5.6, and 10.6 $\mu$g/g creatinine in Arizona, Oklahoma, and the Dakotas, respectively. Increasing baseline arsenic concentrations were associated with female sex, lower educational attainment, lower LDL cholesterol level, higher eGFR, increased prevalence of never-smokers, and increased prevalence of diabetes (**Table 3**).

Baseline urinary arsenic concentrations were prospectively associated with cardiovascular disease mortality and incidence (**Tables 1** and **2** [model 2]). The fully adjusted hazard ratios for cardiovascular disease, coronary heart disease, and stroke mortality when the highest and lowest quartiles of urinary arsenic concentrations were compared were 1.65 (CI, 1.20 to 2.27; $P$ for trend < 0.001), 1.71 (CI, 1.19 to 2.44; $P$ for trend < 0.001), and 3.03 (CI, 1.08 to 8.50; $P$ for trend = 0.061), respectively (**Table 1**

[model 2]). For incident cardiovascular disease, coronary heart disease, and stroke, the corresponding hazard ratios were 1.32 (CI, 1.09 to 1.59; *P* for trend = 0.002), 1.30 (CI, 1.04 to 1.62; *P* for trend = 0.006), and 1.47 (CI, 0.97 to 2.21; *P* for trend = 0.032), respectively (**Table 2** [model 2]). Further adjustment for hypertension, diabetes, eGFR, and especially albuminuria (**Tables 1** and **2** [models 3 and 4]) attenuated the associations. Based on model 2, the incidence rates per 10 000 person-years for increasing arsenic quartiles were 49 (CI, 38 to 61), 56 (CI, 44 to 68), 62 (CI, 50 to 75), and 82 (CI, 66 to 97) for cardiovascular disease mortality and 189 (CI, 164 to 214), 214 (CI, 189 to 239), 198 (CI, 174 to 223), and 238 (CI, 219 to 279) for cardiovascular disease incidence. The dose–response relationships of arsenic concentrations with cardiovascular disease and coronary heart disease incidence and mortality were statistically significant (**Tables 1** and **2**), with no significant departures from linearity (**Figure 1**). For stroke incidence and mortality, the dose–response relationship was positive but not statistically significant (**Tables 1** and **2** and **Appendix Figure 2**, available at www.annals.org).

For cardiovascular and coronary heart disease mortality, the associations were positive for all subgroups but stronger among participants from Arizona, those with diabetes, and those with proportions of DMA above the median (**Appendix Figure 3**, available at www.annals.org). For cardiovascular and coronary heart disease incidence, the associations with urinary arsenic were stronger among women, never-smokers, participants from Arizona, participants with diabetes, and participants with proportions of DMA above the median (**Appendix Figures 4** and **5**, available at www.annals.org). For stroke incidence, the associations were consistent across the subgroups evaluated (**Appendix Figure 6**, available at www.annals.org). In dose–response analyses, the associations of arsenic with cardiovascular disease mortality and incidence were stronger in participants from Arizona (**Figure 2**) and those with diabetes (**Figure 3**).

Urinary arsenobetaine was not associated with any of the cardiovascular end points (data not shown). The fully adjusted hazard ratios for cardiovascular disease mortality and incidence were 0.93 (CI, 0.83 to 1.04) and 0.95 (CI,

*Table 2.* **Incident Cardiovascular End Points (Fatal and Nonfatal), by Urinary Arsenic Concentration* (*n* = 3575)**

| Variable | Quartile of Inorganic Plus Methylated Arsenic Species (μg/g creatinine) | | | | 75th vs. 25th Percentile‡ | *P* Value for Trend§ |
|---|---|---|---|---|---|---|
| | <5.8 (4.2)† | 5.8–9.7 (7.5)† | 9.8–15.7 (12.4)† | >15.7 (21.8)† | | |
| **Cardiovascular disease incidence** | | | | | | |
| Cases, *n* | 265 | 297 | 291 | 331 | 1184 | – |
| Person-years | 12 146 | 11 701 | 11 305 | 10 586 | 45 738 | – |
| Hazard ratio (95% CI)‖ | | | | | | |
| Model 1¶ | 1 (referent) | 1.11 (0.94–1.32) | 1.04 (0.87–1.25) | 1.30 (1.08–1.56) | 1.15 (1.04–1.26) | 0.004 |
| Model 2** | 1 (referent) | 1.14 (0.95–1.35) | 1.05 (0.87–1.26) | 1.32 (1.09–1.59) | 1.16 (1.05–1.28) | 0.002 |
| Model 3†† | 1 (referent) | 1.13 (0.95–1.34) | 1.02 (0.84–1.23) | 1.24 (1.02–1.50) | 1.14 (1.03–1.26) | 0.008 |
| Model 4‡‡ | 1 (referent) | 1.11 (0.93–1.32) | 0.97 (0.80–1.17) | 1.09 (0.90–1.33) | 1.07 (0.97–1.18) | 0.168 |
| **Coronary heart disease incidence** | | | | | | |
| Cases, *n* | 202 | 206 | 197 | 241 | 846 | – |
| Person-years | 12 447 | 12 136 | 11 805 | 11 075 | 47 463 | – |
| Hazard ratio (95% CI)‖ | | | | | | |
| Model 1¶ | 1 (referent) | 0.98 (0.81–1.20) | 0.88 (0.71–1.10) | 1.18 (0.95–1.46) | 1.11 (1.00–1.25) | 0.058 |
| Model 2** | 1 (referent) | 1.05 (0.86–1.28) | 0.95 (0.77–1.19) | 1.30 (1.04–1.62) | 1.17 (1.05–1.32) | 0.006 |
| Model 3†† | 1 (referent) | 1.04 (0.85–1.28) | 0.93 (0.74–1.15) | 1.21 (0.97–1.52) | 1.16 (1.03–1.30) | 0.016 |
| Model 4‡‡ | 1 (referent) | 1.03 (0.84–1.26) | 0.88 (0.70–1.10) | 1.08 (0.86–1.35) | 1.09 (0.97–1.23) | 0.155 |
| **Stroke incidence** | | | | | | |
| Cases, *n* | 55 | 75 | 62 | 72 | 264 | – |
| Person-years | 13 375 | 13 117 | 12 435 | 11 741 | 50 667 | – |
| Hazard ratio (95% CI)‖ | | | | | | |
| Model 1¶ | 1 (referent) | 1.11 (0.78–1.59) | 1.25 (0.84–1.85) | 1.64 (1.10–2.44) | 1.32 (1.09–1.60) | 0.005 |
| Model 2** | 1 (referent) | 1.18 (0.82–1.69) | 1.16 (0.77–1.72) | 1.47 (0.97–2.21) | 1.24 (1.02–1.52) | 0.032 |
| Model 3†† | 1 (referent) | 1.16 (0.81–1.66) | 1.11 (0.74–1.66) | 1.32 (0.87–2.00) | 1.21 (0.98–1.48) | 0.074 |
| Model 4‡‡ | 1 (referent) | 1.09 (0.76–1.57) | 1.07 (0.72–1.60) | 1.18 (0.77–1.79) | 1.14 (0.93–1.41) | 0.21 |

* Sum of inorganic and methylated arsenic species (dimethylarsinate and monomethylarsonate).
† Range (median).
‡ Comparison of the 75th and 25th percentiles (interquartile range) of the sum of inorganic and methylated urinary arsenic concentrations (15.7 vs. 5.8 μg/g creatinine).
§ Obtained from Cox proportional hazards models with log-transformed arsenic as a continuous variable.
‖ Adjustment for systolic blood pressure and hypertension medication use instead of hypertension and for hemoglobin $A_{1c}$ levels instead of diabetes produced similar results (data not shown).
¶ Stratified by study center and adjusted for age (age as time metric and age at baseline were treated as staggered entries).
** Further adjusted for sex, education (none, some, or high school), smoking status (never, former, or current), body mass index (kg/m$^2$), and low-density lipoprotein cholesterol level (mg/dL).
†† Further adjusted for hypertension (yes or no), diabetes (yes or no), and estimated glomerular filtration rate (mL/min/1.73 m$^2$).
‡‡ Further adjusted for albuminuria (yes or no).

*Table 3.* **Baseline Characteristics of Study Participants, Overall and by Urinary Arsenic Concentration* ($n$ = 3575)**

| Characteristic | Overall | Quartile of Inorganic Plus Methylated Arsenic Species ($\mu$g/g creatinine) | | | | *P* Value for Trend‡ |
|---|---|---|---|---|---|---|
| | | <5.8 (4.2)† | 5.8–9.7 (7.5)† | 9.8–15.7 (12.4)† | >15.7 (21.8)† | |
| Mean age (SD), *y* | 56.0 (8.0) | 56.3 (8.2) | 55.5 (7.9) | 56.2 (7.9) | 55.9 (7.9) | 0.77 |
| Female, % | 60.2 | 51.9 | 61.3 | 61.0 | 66.9 | <0.001 |
| State, % | | | | | | |
| Arizona | 34.3 | 7.5 | 25.7 | 44.3 | 59.9 | <0.001 |
| North/South Dakota | 32.5 | 22.7 | 35.2 | 38.6 | 33.6 | <0.001 |
| Oklahoma | 33.2 | 69.9 | 39.1 | 17.2 | 6.5 | <0.001 |
| Less than high school education, % | 46.6 | 33.1 | 38.7 | 52.1 | 62.3 | <0.001 |
| Mean BMI (SD), *kg/m²* | 30.8 (6.3) | 30.5 (5.7) | 31.4 (6.3) | 30.7 (6.2) | 30.7 (6.8) | 0.72 |
| Smoking, % | | | | | | |
| Never | 32.8 | 30.5 | 32.1 | 33.2 | 35.3 | 0.026 |
| Former | 33.6 | 35.2 | 34.7 | 32.5 | 31.9 | 0.088 |
| Current | 33.7 | 34.4 | 33.1 | 34.3 | 32.8 | 0.61 |
| Mean LDL cholesterol level (SD) | | | | | | |
| *mmol/L* | 3.0 (0.9) | 3.1 (0.8) | 3.1 (0.9) | 2.9 (0.9) | 2.9 (0.9) | <0.001 |
| *mg/dL* | 116.1 (33.7) | 121.6 (32.4) | 118.0 (33.4) | 113.0 (33.8) | 111.3 (34.4) | <0.001 |
| Hypertension, % | 37.3 | 36.9 | 35.4 | 38.5 | 38.4 | 0.31 |
| Diabetes, % | 48.5 | 36.9 | 45.0 | 49.6 | 62.4 | <0.001 |
| Mean eGFR (SD), *mL/min/1.73 m²* | 97.9 (17.8) | 94.9 (17.3) | 97.7 (16.7) | 98.1 (17.8) | 101.0 (18.9) | <0.001 |
| Albuminuria, % | 29.0 | 17.1 | 23.2 | 29.9 | 46.1 | <0.001 |

BMI = body mass index; eGFR = estimated glomerular filtration rate; LDL = low-density lipoprotein.
* Sum of inorganic and methylated arsenic species (dimethylarsinate and monomethylarsonate).
† Range (median).
‡ Obtained from linear or logistic models with arsenic quartile entered as a continuous variable.

0.88 to 1.01), respectively, when the 25th and 75th percentiles of arsenobetaine concentrations were compared.

In the subset of participants with detectable inorganic arsenic, MMA, and DMA ($n$ = 3381), hazard ratios for incident cardiovascular disease when an interquartile range of the proportions of inorganic arsenic, MMA, and DMA was compared were 0.91 (CI, 0.84 to 0.99), 0.98 (CI, 0.90 to 1.07), and 1.04 (CI, 0.96 to 1.12), respectively, after adjustment for sex, education, smoking, body mass index, LDL cholesterol level, and the sum of inorganic and methylated arsenic species ($\mu$g/g creatinine). We found no associations between any of the biomarkers of arsenic metabolism and other study end points (not shown).

## DISCUSSION

Exposure to low to moderate levels of inorganic arsenic, as measured in urine, was prospectively associated with fatal and nonfatal cardiovascular disease in a sample of rural American Indians with a high burden of diabetes and cardiovascular disease. The associations persisted after adjustment for sociodemographic factors, smoking, and lipid levels and were attenuated with further adjustment for hypertension, diabetes, and measures of kidney disease, variables that could be in the causal pathway. The associations were largely similar for coronary heart disease and stroke and were stronger for cardiovascular mortality than for cardiovascular incidence. Urinary arsenobetaine, an organic arsenical that is found in seafood and is believed to be nontoxic, was not associated with cardiovascular disease. Overall, our findings support an association between long-term exposure to low to moderate levels of inorganic arsenic and incident cardiovascular disease.

The adverse cardiovascular effects of long-term exposure to high arsenic levels in drinking water (>100 $\mu$g/L) have long been recognized (34). In early case reports, exposure to high arsenic levels was associated with peripheral artery disease in southwestern Taiwan and in German vintners (35) and with myocardial infarction in young adults from Chile (36). Long-term exposure to high levels of arsenic in drinking water was prospectively associated with coronary heart disease mortality in southwestern Taiwan (3). Recently, prospective studies from Bangladesh found that urine and drinking water arsenic concentrations were associated with increased cardiovascular disease and coronary heart disease mortality (5, 6). Evidence from multiple countries with different ethnic backgrounds supports a causal association between long-term exposure to high levels of arsenic and cardiovascular disease (12, 13).

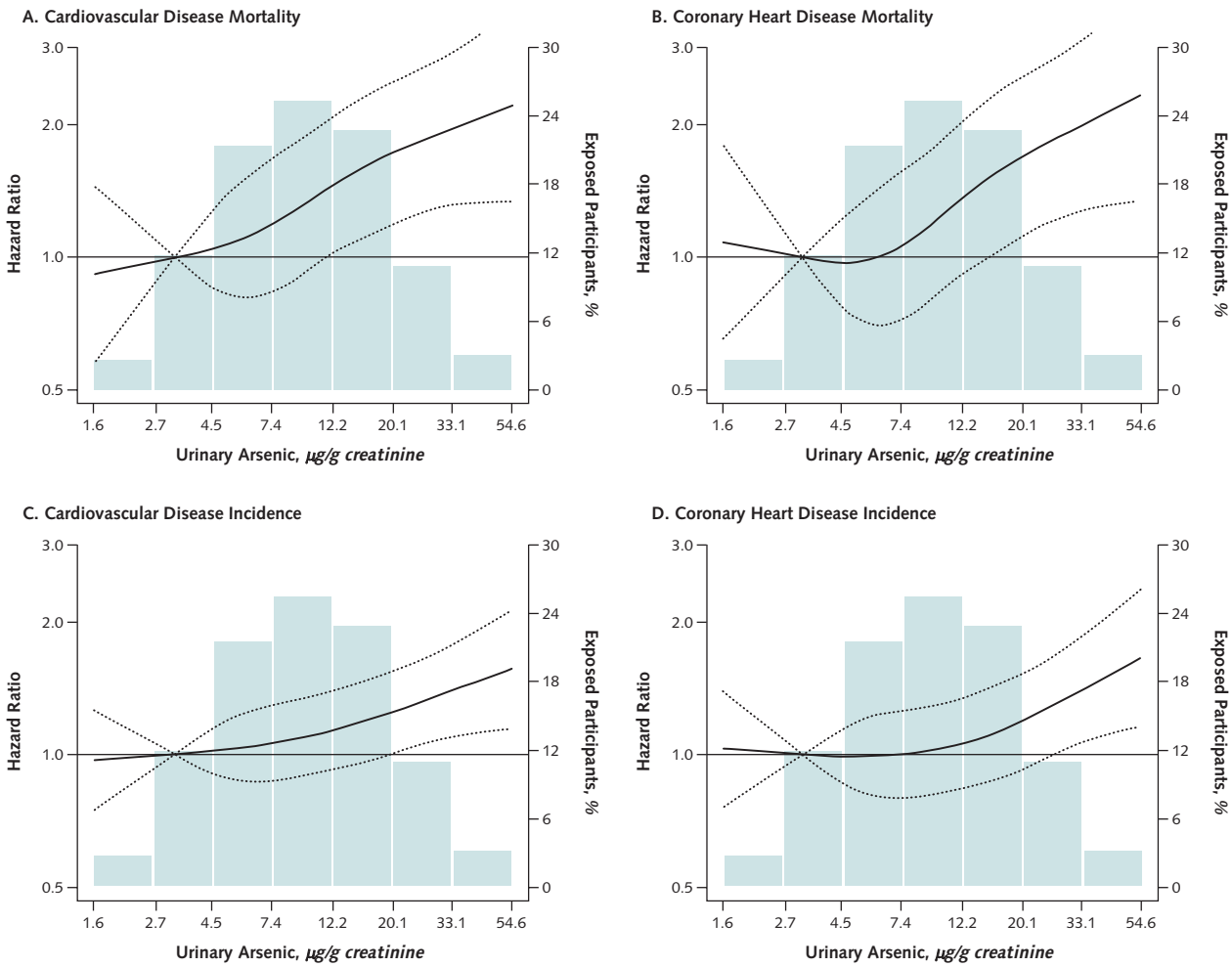
Less is known about the cardiovascular effects of arsenic at levels less than 100 $\mu$g/L in drinking water. Some (37–44), but not all (45, 46), studies have found modestly increased cardiovascular risks, although most of these studies had important limitations, including ecological designs, limited exposure and outcome assessment, and lack of adjustment for cardiovascular risk factors. In 2 recent prospective studies from Bangladesh, the hazard ratios for low to moderate arsenic exposure categories (arsenic concentrations in drinking water of 12.1 to 62 $\mu$g/L vs. ≤12 $\mu$g/L [6] and 10 to 49 $\mu$g/L vs. <10 $\mu$g/L [5]) and cardiovas-

cular mortality were supportive of an association but not statistically significant. Thus, our study provides important novel data in a Western population with high background cardiovascular risk.

Experimental studies also support the role of arsenic in cardiovascular disease. Animals exposed to arsenic were more likely to develop atherosclerotic plaque than unexposed animals (47–49). Potential mechanisms for arsenic-related atherosclerosis include endothelial dysfunction, smooth-muscle proliferation, angiogenesis and apoptosis, vascular injury, and platelet aggregation (50, 51). In addition, arsenic upregulates inflammation, disrupts lipid metabolism, and increases lipid oxidation (50, 51). Arsenic-related cardiovascular disease could also be mediated by other cardiovascular risk factors, including hypertension (52), diabetes (53, 54), and kidney disease (55–57).

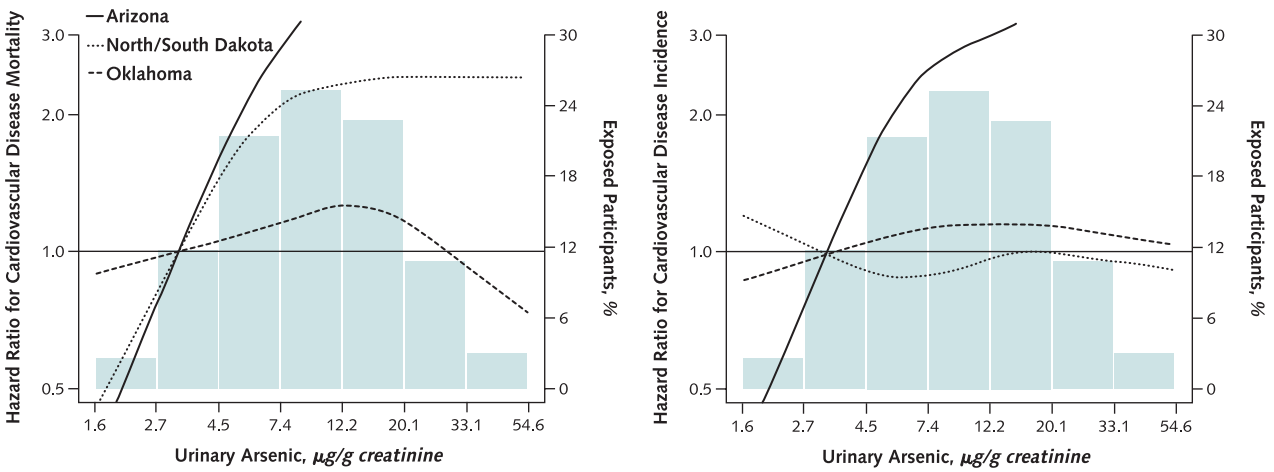
Our subgroup analyses must be interpreted cautiously. Some studies have reported differences by smoking status (6), urinary arsenic metabolic patterns (4, 33), and genetic factors (58). In our study, we found stronger associations among never-smokers, contrary to a stronger association among current smokers in a prospective cohort study in Bangladesh (6). Susceptibility to arsenic toxicity may also differ by sex (59), although previous studies of arsenic and cardiovascular disease found no marked differences by sex (2, 10, 35, 40, 45). We found a stronger association among women, although arsenic was associated with an increased risk for cardiovascular disease in both sexes. In studies eval-

***Figure 1.*** **Hazard ratios for cardiovascular disease and coronary heart disease incidence and mortality, by urinary arsenic concentration ($n$ = 3575).**




Solid lines represent adjusted hazard ratios based on restricted quadratic splines for the log-transformed sum of inorganic and methylated arsenic species, with knots at the 10th, 50th, and 90th percentiles (3.8, 9.7, and 24.0 μg/g creatinine, respectively). The dotted lines represent upper and lower 95% CIs. The reference was set at the 10th percentile of the arsenic distribution (3.8 μg/g creatinine). Adjustment factors were the same as those for model 2 in **Tables 1** and **2**. The bars represent a histogram of urinary arsenic distribution among participants (the extreme tails of the histogram were truncated because only 1 participant had a urinary arsenic level <1.6 μg/g creatinine and 31 had a level >54.6 μg/g creatinine).

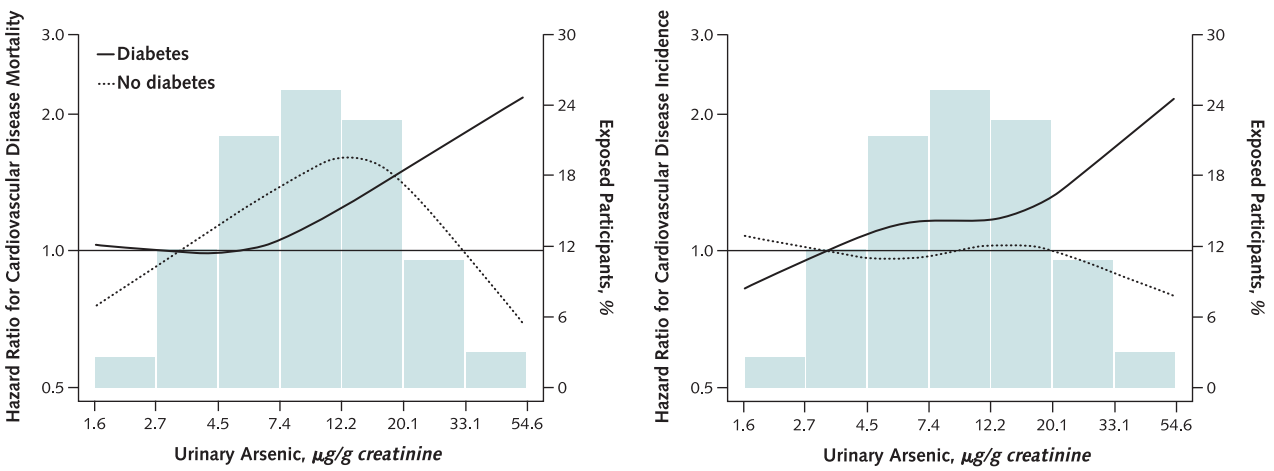
*Figure 2.* **Hazard ratios for cardiovascular disease incidence and mortality, stratified by study region.**




The lines represent adjusted hazard ratios based on restricted quadratic splines for the log-transformed sum of inorganic and methylated arsenic species, with knots at the 10th, 50th, and 90th percentiles (3.8, 9.7, and 24.0 μg/g creatinine, respectively). The reference was set at the 10th percentile of the arsenic distribution (3.8 μg/g creatinine). Adjustment factors were the same as those for model 2 in **Tables 1** and **2**. The bars represent a histogram of urinary arsenic distribution among participants (the extreme tails of the histogram were truncated because only 1 participant had a urinary arsenic level <1.6 μg/g creatinine and 31 had urinary arsenic levels >54.6 μg/g creatinine).

uating the health effects of arsenic metabolism conducted in Taiwan, individuals with a higher proportion of MMA or lower proportion of DMA in urine had a higher risk for peripheral artery disease (4), carotid atherosclerosis (33), and hypertension (60). In addition, the association between arsenic exposure and cardiovascular disease was stronger among participants with a higher proportion of MMA (4, 33). In our study, which examined exposure to low to moderate arsenic levels, we found no association between arsenic metabolic patterns in urine and cardiovascular risk. The association between arsenic and cardiovascular disease, on the other hand, was stronger among participants with a higher proportion of DMA. Additional research is needed to evaluate the effect modification of

*Figure 3.* **Hazard ratios for cardiovascular disease incidence and mortality, stratified by diabetes status at baseline.**




The lines represent adjusted hazard ratios based on restricted quadratic splines for the log-transformed sum of inorganic and methylated arsenic species, with knots at the 10th, 50th, and 90th percentiles (3.8, 9.7, and 24.0 μg/g creatinine, respectively). The reference was set at the 10th percentile of the arsenic distribution (3.8 μg/g creatinine). Adjustment factors were the same as those for model 2 in **Tables 1** and **2**. The bars represent a histogram of urinary arsenic distribution among participants (the extreme tails of the histogram were truncated because only 1 participant had a urinary arsenic level <1.6 μg/g creatinine and 31 had urinary arsenic levels >54.6 μg/g creatinine).

arsenic exposure by arsenic metabolism in populations exposed to low to moderate arsenic levels.

We also found differences in the association between arsenic and cardiovascular disease end points by study region and diabetes status, subgroups that have not been evaluated before. The stronger association in Arizona than in other regions could be related to higher arsenic exposure, residual confounding, effect modification by other co-exposures, differences in access to care and surveillance methods across the 3 study regions, or gene–arsenic interactions. In a previous linkage study in the Strong Heart Family Study, we found different peaks across the genome associated with arsenic metabolism measures across study regions (61). We found stronger associations among participants with diabetes than among those without it. Arsenic has been consistently associated with diabetes in populations exposed to high levels in drinking water (54), and recent prospective studies from the United States that examined exposure to low to moderate levels, including among American Indians in the Southwest, support arsenic exposure as a diabetes risk factor (62, 63). Diabetes could be in the causal pathway between arsenic exposure and cardiovascular disease. Alternatively, it could confound the association between arsenic exposure (as measured in urine) and cardiovascular disease. In the Strong Heart Study, baseline urinary arsenic levels were associated with poor diabetes control (53). However, further adjustment for hemoglobin $A_{1c}$ levels in this analysis produced similar results. Overall, the stronger association between arsenic and cardiovascular disease among participants with diabetes must be interpreted cautiously and requires replication in other populations. Hypertension (64) and kidney disease (55–57) could also be in the causal pathway. Including these variables in the models could have resulted in overadjustment.

Strengths of this study include high-quality data collection methods and surveillance for cardiovascular disease outcomes over a long follow-up (20) and rigorous laboratory methods for measuring concentrations of urinary arsenic species (24). Urinary arsenic measurements integrate all sources of exposure at the individual level, including water and food, and are an excellent biomarker of internal dose (1, 25, 26). This study also had several limitations. We measured urinary arsenic levels in a single sample at baseline, and individual levels in drinking water were unavailable. The temporal stability of arsenic levels in public and private drinking water and in urine has been shown in several studies in the United States (17, 65–67). Several studies have also shown consistent associations with cardiovascular end points when arsenic levels measured in water and urine were compared (4, 6). Other limitations include the possibility of residual confounding (for example, access to care or geographic factors), overadjustment for variables that could be in the causal pathway (diabetes, hypertension, or kidney disease), and exposure and outcome misclassification.

More than 100 million persons worldwide are exposed to arsenic levels in drinking water above the World Health Organization standard of 10 μg/L (68, 69). In 2001, the U.S. Environmental Protection Agency estimated that 13 million Americans were exposed to levels above 10 μg/L (14). Many more millions are exposed to arsenic through food, although no standards for inorganic arsenic in food currently exist. Given the large population exposed, even a modest increased risk for cardiovascular disease due to arsenic could have important public health implications. Cardiovascular disease is the leading cause of death in the United States, with rates among American Indians exceeding those of the general U.S. population (70). Arsenic mitigation could reduce the burden of cardiovascular disease. Discussions to revise the current U.S. Environmental Protection Agency safety standard for arsenic in drinking water should quantitatively consider the evidence supporting the cardiovascular disease effects of exposure to low to moderate levels of arsenic.

In conclusion, exposure to low to moderate levels of inorganic arsenic, as measured in urine, was prospectively associated with increased fatal and nonfatal cardiovascular disease in a U.S. population with a high burden of diabetes and cardiovascular disease. These findings support the importance of exposure to low to moderate arsenic levels as a cardiovascular risk factor with no apparent threshold.

From Johns Hopkins Bloomberg School of Public Health and Johns Hopkins Medical Institutions, Baltimore, Maryland; National Center for Cardiovascular Research, Madrid, Spain; MedStar Health Research Institute, Hyattsville, Maryland; Georgetown University and Georgetown-Howard Universities Center for Clinical and Translational Science, Washington, DC; New York Presbyterian Hospital/Weill Medical College of Cornell Medical Center, New York, New York; Missouri Breaks Industries Research, Timber Lake, South Dakota; and Karl-Franzens University, Graz, Austria.

**Note:** Ms. Moon and Dr. Navas-Acien had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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**Reproducible Research Statement:** *Study protocol:* The original study proposal funded by the National Institutes of Health and the manuscript proposal approved by the Strong Heart Study Publications and Presentations Committee are available from Dr. Navas-Acien (e-mail, anavas@jhsph.edu). *Statistical code:* Available from Dr. Navas-Acien (e-mail, anavas@jhsph.edu). *Data set:* As with other National Heart, Lung, and Blood Institute cohorts, the Strong Heart Study data are not publicly available. Outside investigators may apply to use the data generated according to the established protocols for Strong Heart Study Resource and

Data Sharing, including community approval, through formal application (http://strongheart.ouhsc.edu/datarequest.html).

**Requests for Single Reprints:** Ana Navas-Acien, MD, PhD, Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health Sciences, 615 North Wolfe Street, Room W7513D, Baltimore, MD 21205; e-mail, anavas@jhsph.edu.

Current author addresses and author contributions are available at www.annals.org.

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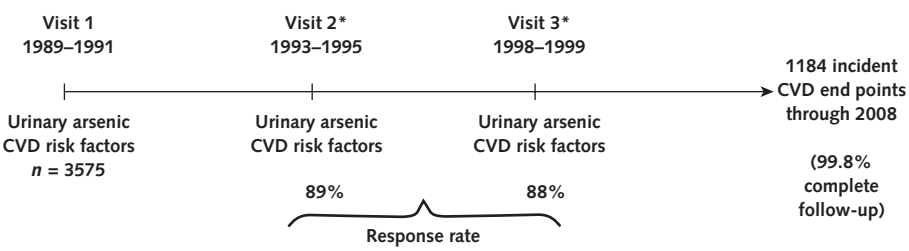
**Current Author Addresses:** Ms. Moon: Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health Sciences, 615 North Wolfe Street, Room W7604, Baltimore, MD 21205.
Dr. Guallar: Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology, 2024 East Monument Street, Room 2-639, Baltimore, MD 21205.
Drs. Umans and Howard: MedStar Health Research Institute, 6525 Belcrest Road, Suite 700, Hyattsville, MD 20782.
Dr. Devereux: Weill Medical College of Cornell Medical Center, 520 East 70th Street, Room K-4, New York, NY 10021.
Dr. Best: 1935 188th Avenue NW, Watford City, ND 58854.
Drs. Francesconi and Goessler: Institute for Chemistry–Analytical Chemistry, Karl Franzens University, Stremayrgasse 16/3, Stock, 8010 Graz, Austria.
Mr. Pollak: Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health Sciences, 615 North Wolfe Street, Room W7508A, Baltimore, MD 21205.
Dr. Silbergeld: Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health Sciences, 615 North Wolfe Street, Room E6644, Baltimore, MD 21205.
Dr. Navas-Acien: Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health Sciences, 615 North Wolfe Street, Room W7513D, Baltimore, MD 21205.

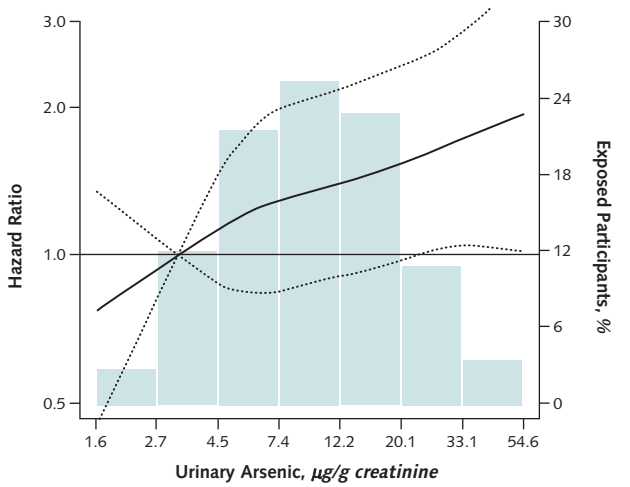
**Author Contributions:** Conception and design: E. Guallar, J. Pollak, B.V. Howard, A. Navas-Acien.
Analysis and interpretation of the data: K.A. Moon, E. Guallar, J.G. Umans, K.A. Francesconi, W. Goessler, J. Pollak, E.K. Silbergeld, B.V. Howard, A. Navas-Acien.
Drafting of the article: K.A. Moon, L.G. Best, A. Navas-Acien.
Critical revision of the article for important intellectual content: K.A. Moon, E. Guallar, J.G. Umans, R.B. Devereux, J. Pollak, E.K. Silbergeld, B.V. Howard, A. Navas-Acien.
Final approval of the article: E. Guallar, J.G. Umans, R.B. Devereux, L.G. Best, W. Goessler, J. Pollak, E.K. Silbergeld, B.V. Howard, A. Navas-Acien.
Provision of study materials or patients: J.G. Umans, L.G. Best.
Statistical expertise: E. Guallar, J. Pollak, A. Navas-Acien.
Obtaining of funding: E. Guallar, L.G. Best, B.V. Howard, A. Navas-Acien.
Administrative, technical, or logistic support: J.G. Umans, L.G. Best, W. Goessler, A. Navas-Acien.
Collection and assembly of data: L.G. Best, W. Goessler, J. Pollak, B.V. Howard, A. Navas-Acien.

*Appendix Figure 1.* **Strong Heart Study clinic visits, follow-up, and data used in the present study.**



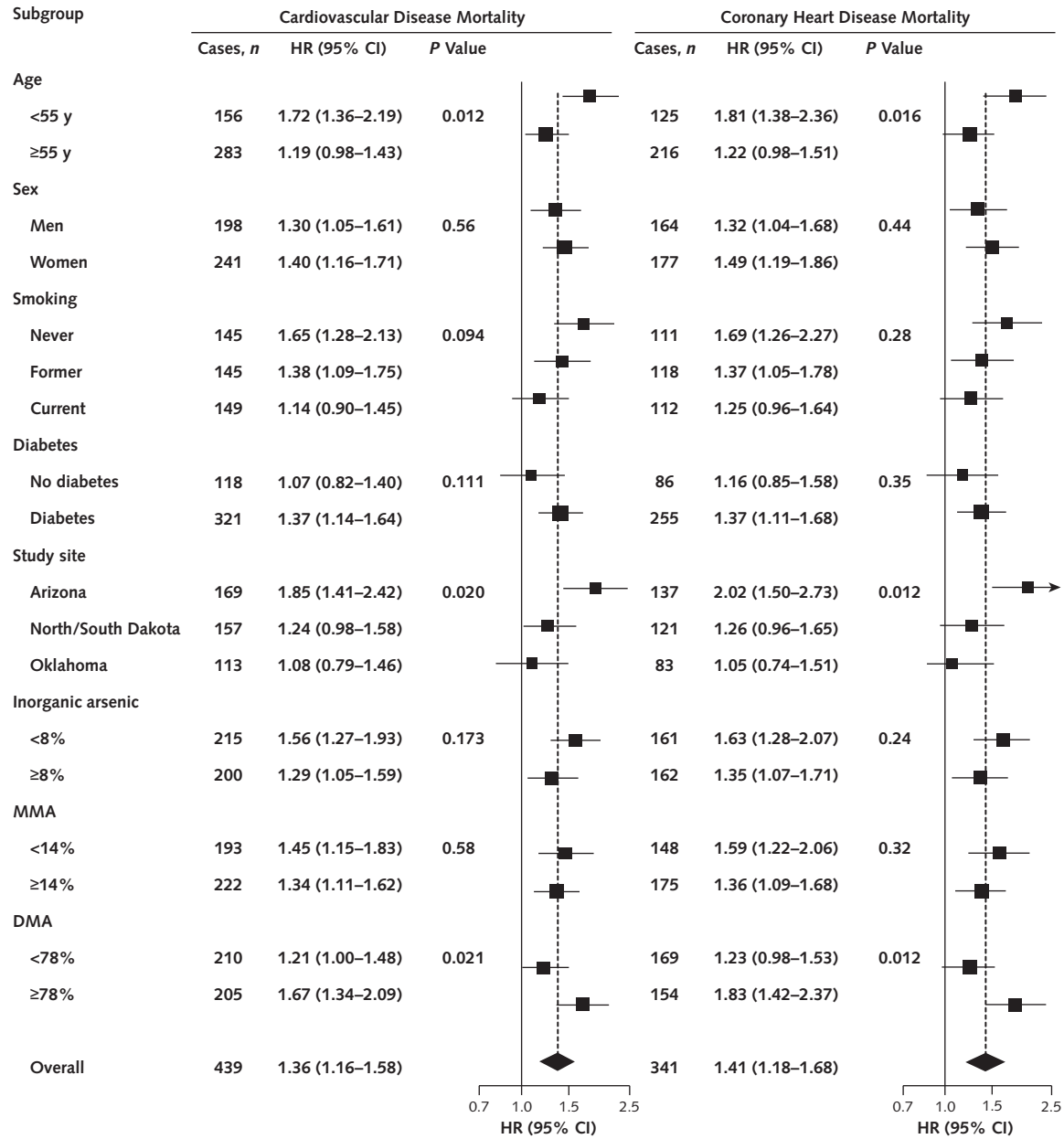

CVD = cardiovascular disease.
* Urinary arsenic concentrations were available for only 380 participants.

*Appendix Figure 2.* **Hazard ratios for stroke incidence, by urinary arsenic concentration ($n$ = 3575).**




The solid line represents the adjusted hazard ratio based on restricted quadratic splines for the log-transformed sum of inorganic and methylated arsenic species, with knots at the 10th, 50th, and 90th percentiles (3.8, 9.7, and 24.0 μg/g creatinine, respectively). The dotted lines represent upper and lower 95% CIs. The reference was set at the 10th percentile of the arsenic distribution (3.8 μg/g creatinine). Adjustment factors were the same as those for model 2 in **Tables 1** and **2**. The bars represent a histogram of urinary arsenic distribution among participants (the extreme tails of the histogram were truncated because only 1 participant had a urinary arsenic level <1.6 μg/g creatinine and 31 had urinary arsenic levels >54.6 μg/g creatinine).

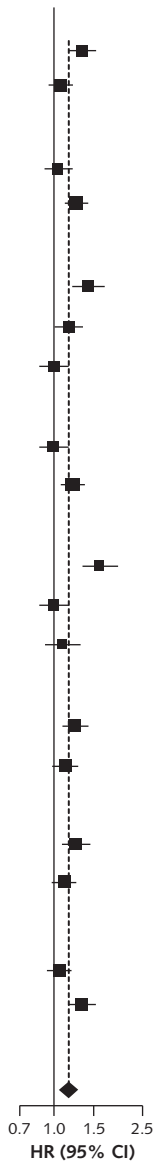
*Appendix Figure 3.* **HRs and 95% CIs for cardiovascular disease and coronary heart disease mortality when an interquartile range of urinary arsenic concentrations is compared, by participant characteristics at baseline ($n$ = 3575).**




HRs for cardiovascular disease and coronary heart disease mortality were stratified by each subgroup of interest, and associated $P$ values for interaction were obtained from stratified Cox proportional hazards models with log-transformed arsenic (sum of inorganic and methylated arsenic species) as a continuous variable, adjusted for the same covariates as those in model 2 in **Tables 1** and **2**. The interquartile range of urinary arsenic concentrations was 5.8 to 15.7 $\mu$g/g creatinine. For cardiovascular disease and coronary heart disease mortality HRs by methylation indices (below and above the median proportions of inorganic arsenic, MMA, and DMA), the data set was restricted to participants with detectable inorganic arsenic, MMA, and DMA concentrations ($n$ = 3381). For this subset, the corresponding urinary arsenic interquartile range was 6.1 to 16.2 $\mu$g/g creatinine. DMA = dimethylarsinate; HR = hazard ratio; MMA = monomethylarsonate.

*Appendix Figure 4.* **HRs and 95% CIs for cardiovascular disease incidence when an interquartile range of urinary arsenic concentrations is compared, by participant characteristics at baseline ($n$ = 3575).**

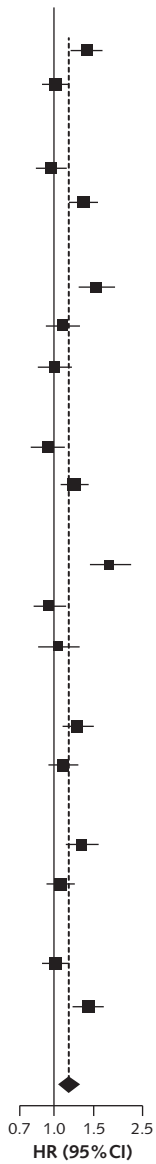
| Subgroup | Cases, *n* | HR (95% CI) | *P* Value |
|---|---|---|---|
| Age | | | |
| <55 y | 470 | 1.33 (1.16–1.53) | 0.011 |
| ≥55 y | 714 | 1.06 (0.94–1.20) | |
| Sex | | | |
| Men | 501 | 1.04 (0.90–1.19) | 0.027 |
| Women | 683 | 1.25 (1.11–1.40) | |
| Smoking | | | |
| Never | 362 | 1.42 (1.21–1.67) | 0.003 |
| Former | 401 | 1.16 (1.00–1.34) | |
| Current | 421 | 1.00 (0.86–1.15) | |
| Diabetes | | | |
| No diabetes | 425 | 0.99 (0.86–1.15) | 0.025 |
| Diabetes | 759 | 1.21 (1.07–1.36) | |
| Study site | | | |
| Arizona | 378 | 1.60 (1.34–1.92) | <0.001 |
| North/South Dakota | 471 | 1.00 (0.86–1.15) | |
| Oklahoma | 335 | 1.09 (0.91–1.31) | |
| Inorganic arsenic | | | |
| <8% | 575 | 1.24 (1.09–1.42) | 0.23 |
| ≥8% | 549 | 1.12 (0.98–1.27) | |
| MMA | | | |
| <14% | 538 | 1.25 (1.09–1.44) | 0.151 |
| ≥14% | 586 | 1.11 (0.98–1.25) | |
| DMA | | | |
| <78% | 562 | 1.06 (0.93–1.19) | 0.007 |
| ≥78% | 562 | 1.33 (1.16–1.52) | |
| Overall | 1184 | 1.16 (1.05–1.28) | |




HRs for cardiovascular disease incidence (fatal and nonfatal events) were stratified by each subgroup of interest, and associated *P* values for interaction were obtained from stratified Cox proportional hazards models with log-transformed arsenic (sum of inorganic and methylated arsenic species) as a continuous variable, adjusted for the same covariates as those in model 2 in the main analysis. The interquartile range of urinary arsenic concentrations was 5.8 to 15.7 μg/g creatinine. For cardiovascular disease incidence HRs by methylation indices (below and above median proportions of inorganic arsenic, MMA, and DMA), the data set was restricted to participants with detectable inorganic arsenic, MMA, and DMA concentrations ($n$ = 3381). For this subset, the corresponding urinary arsenic interquartile range was 6.1 to 16.2 μg/g creatinine. DMA = dimethylarsinate; HR = hazard ratio; MMA = monomethylarsonate.

*Appendix Figure 5.* **HRs and 95% CIs for coronary heart disease incidence when an interquartile range of urinary arsenic concentrations is compared, by participant characteristics at baseline ($n$ = 3575).**

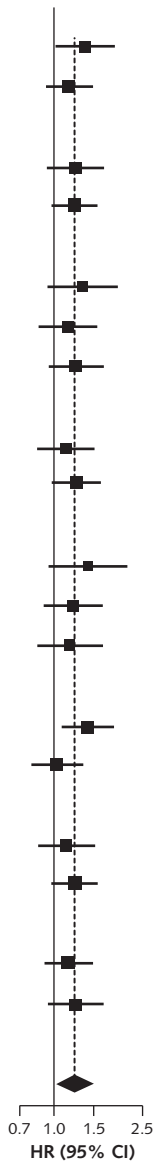
| Subgroup | Cases, *n* | HR (95% CI) | *P* Value |
|---|---|---|---|
| Age | | | |
| <55 y | 362 | 1.40 (1.19–1.64) | 0.002 |
| ≥55 y | 484 | 1.02 (0.88–1.18) | |
| Sex | | | |
| Men | 397 | 0.97 (0.83–1.14) | <0.001 |
| Women | 449 | 1.36 (1.18–1.57) | |
| Smoking | | | |
| Never | 254 | 1.55 (1.29–1.87) | 0.001 |
| Former | 291 | 1.10 (0.92–1.13) | |
| Current | 301 | 1.01 (0.85–1.20) | |
| Diabetes | | | |
| No diabetes | 294 | 0.94 (0.79–1.12) | 0.009 |
| Diabetes | 552 | 1.24 (1.08–1.43) | |
| Study site | | | |
| Arizona | 273 | 1.79 (1.45–2.21) | <0.001 |
| North/South Dakota | 335 | 0.96 (0.81–1.14) | |
| Oklahoma | 238 | 1.05 (0.85–1.31) | |
| Inorganic arsenic | | | |
| <8% | 396 | 1.28 (1.10–1.50) | 0.154 |
| ≥8% | 409 | 1.11 (0.95–1.29) | |
| MMA | | | |
| <14% | 379 | 1.34 (1.14–1.58) | 0.027 |
| ≥14% | 426 | 1.07 (0.93–1.24) | |
| DMA | | | |
| <78% | 416 | 1.02 (0.88–1.18) | 0.001 |
| ≥78% | 389 | 1.43 (1.21–1.68) | |
| Overall | 846 | 1.17 (1.05–1.32) | |




HRs for coronary heart disease incidence (fatal and nonfatal events) were stratified by each subgroup of interest, and associated *P* values for interaction were obtained from stratified Cox proportional hazards models with log-transformed arsenic (sum of inorganic and methylated arsenic species) as a continuous variable, adjusted for the same covariates as those in model 2 in the main analysis. The interquartile range of urinary arsenic concentrations was 5.8 to 15.7 μg/g creatinine. For coronary heart disease incidence HRs by methylation indices (below and above median proportions of inorganic arsenic, MMA, and DMA), the data set was restricted to participants with detectable inorganic arsenic, MMA, and DMA concentrations ($n$ = 3381). For this subset, the corresponding urinary arsenic interquartile range was 6.1 to 16.2 μg/g creatinine. DMA = dimethylarsinate; HR = hazard ratio; MMA = monomethylarsonate.

*Appendix Figure 6.* **HRs and 95% CIs for stroke incidence when an interquartile range of urine arsenic concentrations is compared, by participant characteristics at baseline ($n$ = 3575).**

| Subgroup | Cases, *n* | HR (95% CI) | *P* Value |
|---|---|---|---|
| Age | | | |
| <55 y | 99 | 1.38 (1.02–1.88) | 0.37 |
| ≥55 y | 165 | 1.17 (0.92–1.49) | |
| Sex | | | |
| Men | 101 | 1.25 (0.93–1.68) | 0.97 |
| Women | 163 | 1.24 (0.98–1.57) | |
| Smoking | | | |
| Never | 72 | 1.35 (0.94–1.94) | 0.78 |
| Former | 88 | 1.16 (0.85–1.57) | |
| Current | 104 | 1.26 (0.95–1.67) | |
| Diabetes | | | |
| No diabetes | 93 | 1.13 (0.84–1.52) | 0.54 |
| Diabetes | 171 | 1.27 (0.98–1.63) | |
| Study site | | | |
| Arizona | 77 | 1.43 (0.95–2.14) | 0.76 |
| North/South Dakota | 100 | 1.22 (0.90–1.65) | |
| Oklahoma | 87 | 1.18 (0.84–1.67) | |
| Inorganic arsenic | | | |
| <8% | 130 | 1.43 (1.09–1.86) | 0.070 |
| ≥8% | 125 | 1.03 (0.79–1.35) | |
| MMA | | | |
| <14% | 123 | 1.14 (0.85–1.53) | 0.63 |
| ≥14% | 132 | 1.25 (0.98–1.58) | |
| DMA | | | |
| <78% | 130 | 1.16 (0.91–1.49) | 0.66 |
| ≥78% | 125 | 1.26 (0.94–1.67) | |
| Overall | 264 | 1.24 (1.02–1.52) | |




HRs for stroke incidence (fatal and nonfatal events) were stratified by each subgroup of interest, and associated *P* values for interaction were obtained from stratified Cox proportional hazards models with log-transformed arsenic (sum of inorganic and methylated arsenic species) as a continuous variable, adjusted for the same covariates as those in model 2 in the main analysis. The interquartile range of urine arsenic concentrations was 5.8 to 15.7 μg/g creatinine. For stroke incidence HRs by methylation indices (below and above median proportions of inorganic arsenic, MMA, and DMA), the data set was restricted to participants with detectable inorganic arsenic, MMA, and DMA concentrations ($n$ = 3381). For this subset, the corresponding urinary arsenic interquartile range was 6.1 to 16.2 μg/g creatinine. DMA = dimethylarsinate; HR = hazard ratio; MMA = monomethylarsonate.