



Arsenic Exposure, Arsenic Metabolism, and Incident Diabetes in the Strong Heart Study

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OBJECTIVE

Little is known about arsenic metabolism in diabetes development. We investigated the prospective associations of low-moderate arsenic exposure and arsenic metabolism with diabetes incidence in the Strong Heart Study.

RESEARCH DESIGN AND METHODS

A total of 1,694 diabetes-free participants aged 45–75 years were recruited in 1989–1991 and followed through 1998–1999. We used the proportions of urine inorganic arsenic (iAs), monomethylarsonate (MMA), and dimethylarsinate (DMA) over their sum (expressed as iAs%, MMA%, and DMA%) as the biomarkers of arsenic metabolism. Diabetes was defined as fasting glucose ≥ 126 mg/dL, 2-h glucose ≥ 200 mg/dL, self-reported diabetes history, or self-reported use of anti-diabetic medications.

RESULTS

Over 11,263.2 person-years of follow-up, 396 participants developed diabetes. Using the leave-one-out approach to model the dynamics of arsenic metabolism, we found that lower MMA% was associated with higher diabetes incidence. The hazard ratios (95% CI) of diabetes incidence for a 5% increase in MMA% were 0.77 (0.63–0.93) and 0.82 (0.73–0.92) when iAs% and DMA%, respectively, were left out of the model. DMA% was associated with higher diabetes incidence only when MMA% decreased (left out of the model) but not when iAs% decreased. iAs% was also associated with higher diabetes incidence when MMA% decreased. The association between MMA% and diabetes incidence was similar by age, sex, study site, obesity, and urine iAs concentrations.

CONCLUSIONS

Arsenic metabolism, particularly lower MMA%, was prospectively associated with increased incidence of diabetes. Research is needed to evaluate whether arsenic metabolism is related to diabetes incidence per se or through its close connections with one-carbon metabolism.

Humans are exposed to inorganic arsenic (iAs) through drinking water, food, dust, and ambient air (1). Increasing epidemiologic and experimental evidence supports a role for iAs in the development of diabetes (2,3). At high arsenic levels (>150 $\mu\text{g/L}$ in drinking water), evidence from Taiwan and Bangladesh supports an association with diabetes, although most studies are cross-sectional and concerns exist about the measures of arsenic exposure and definition of diabetes used in some studies (2,4). At low-moderate arsenic levels, evidence from Mexico and the

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U.S., including cross-sectional (5,6) and prospective studies (7,8), support the role of arsenic in diabetes development.

Little is known, however, about the association between arsenic metabolism and diabetes. After absorption, iAs (arsenate and arsenite) is methylated, primarily in the liver, to form monomethylated and dimethylated arsenic compounds monomethylarsonate (MMA) and dimethylarsinate (DMA), which are excreted into the urine together with iAs (9,10). Higher MMA% and lower DMA% in urine have been related to an increased risk of cancer (11–13) and cardiovascular disease in studies from Taiwan and Bangladesh (14,15) and may be related to the high toxicity of MMA(III), the trivalent form that is rapidly oxidized to MMA in urine and thus difficult to measure in epidemiologic studies (16,17). DMA is regarded as a less toxic arsenic species because it is more rapidly excreted through the urine than iAs (18,19). DMA(III), however, has been linked to the prevalence of diabetes in cross-sectional studies from Mexico and Bangladesh, although it is also an unstable species in urine (5,20). Higher DMA% and lower MMA% have also been related to obesity in studies from Mexico and the U.S. (21,22), although the temporality of these associations is unclear. Furthermore, arsenic metabolism is tightly connected with one-carbon metabolism (23), which has been implicated in both cancer development and cardiovascular disease (24,25) and may play a role in diabetes (26,27). These findings highlight the need to properly evaluate the role of arsenic methylation profiles in diabetes development.

In this study, we investigated the associations of low-moderate arsenic exposure and arsenic metabolism with diabetes in the Strong Heart Study (SHS). The SHS is a population-based prospective cohort study of cardiometabolic diseases among three American Indian communities in rural Arizona, Oklahoma, and North and South Dakota (28). In participants from Arizona and the Dakotas, drinking water was probably the major source of iAs exposure, whereas in participants from Oklahoma, diet, including rice, flour, and other grains, was probably the main source. Urine arsenic concentrations and measures of arsenic metabolism were stable in SHS participants during follow-up,

supporting the use of urine arsenic as a suitable surrogate for chronic arsenic exposure and metabolism (29). In the SHS, we found that higher iAs exposure was associated with higher diabetes prevalence (6), supporting the need to further investigate the prospective associations between arsenic exposure and metabolism with diabetes incidence.

RESEARCH DESIGN AND METHODS

Study Population

In 1989–1991, the SHS examined 4,549 American Indian men and women aged 45–74 years at baseline enrollment from 13 tribes and communities (30). All community members in Arizona and Oklahoma were invited to participate, whereas a cluster sampling procedure was used in North and South Dakota (31,32). The overall participation rate was 62%. Compared with nonparticipants, participants were similar in age, BMI, and prevalence of self-reported diabetes but were more likely to be female and to have self-reported hypertension (32). Participants were invited to subsequent clinical visits between 1993 and 1995 and between 1998 and 1999 (31,32). The SHS population is stable, with low migration rates due to strong cultural and social links in the community (33). The Indian Health Service, institutional review boards, and participating communities approved the study protocol. All participants provided informed consent.

The prevalence of diabetes in the SHS in 1989–1991 was 50%. For the present study, we used data from participants free of diabetes and with sufficient urine available for arsenic measurements at the baseline visit ($n = 1,986$) (Supplementary Fig. 1). We further excluded 117 participants lost to follow-up or missing both fasting glucose and 2-h plasma glucose data during follow-up, 105 participants with inorganic or methylated arsenic species below the limit of detection because estimating arsenic methylation in these participants is difficult, and 70 participants missing other variables of interest, leaving 1,694 participants for this analysis. Sociodemographic and diabetes risk factors were similar between the present study population and the overall SHS population free of diabetes at baseline (data not shown).

Data Collection

Baseline clinical information comprising a personal interview (sociodemographic, smoking and alcohol status), physical examination (height, weight, waist and hip circumferences, systolic and diastolic blood pressure), fasting blood sample, and spot urine sample was collected by trained and certified personnel using standardized protocols (31). Detailed procedures of clinical and laboratory examinations have been previously described (31). Estimated glomerular filtration rate at baseline was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (34). Participants were asked to fast for 12 h before morning blood sample collection at baseline and in the two subsequent visits. Spot urine samples were also collected in the morning and frozen within 1–2 h of collection. The biospecimens were stored at $\leq -70^{\circ}\text{C}$ before analyses (31).

Diabetes Measurements

Fasting plasma glucose level was determined by hexokinase method. A 2-h, 75-g oral glucose tolerance test was performed in all participants except those on insulin therapy, with poor glycemic control on oral medication, or with a fasting glucose >225 mg/dL as determined by an Accu-Chek II (Baxter Healthcare Corporation, Grand Prairie, TX) glucose meter (31). Glycated hemoglobin was measured by high-performance liquid chromatography (31). Diabetes was defined as a fasting plasma glucose ≥ 126 mg/dL, plasma glucose ≥ 200 mg/dL 2 h after ingestion of a 75-g oral glucose load, self-reported diabetes history, or self-reported use of insulin or oral hypoglycemic medications.

Urine Arsenic

Urine arsenic species were used to assess long-term arsenic exposure. The relative proportions of each urine arsenic species (iAs%, MMA%, and DMA%) standardized by the urine total arsenic concentration were used to approximate individual arsenic methylation profiles. For example, MMA% is determined as urine MMA [(MMA(V) + MMA(III))] concentration divided by urine total iAs concentration (iAs + MMA + DMA). In a subsample of diabetes-free participants with urine arsenic measures repeated over the three study visits ($n = 207$), the individual (single) intraclass correlation coefficient for arsenic measures over a

10-year period was 0.60 for the sum of inorganic and methylated species and 0.55, 0.59, and 0.69 for iAs%, MMA%, and DMA%, respectively, confirming the moderate long-term stability of arsenic exposure and arsenic metabolism in this cohort. Detailed analytic methods and associated quality control procedures for arsenic analysis have been previously published (35). Arsenic speciation can discriminate species directly related to iAs exposure (arsenite, arsenate, MMA, and DMA) from those related to organic arsenicals (arsenobetaine) in seafood, which are generally considered nontoxic (36). Urine concentrations of arsenobetaine and other arsenic cations were very low (median 0.71 [interquartile range 0.41–1.69] $\mu\text{g/g}$ creatinine), confirming that seafood intake was low in this sample and indicating that DMA mainly came from iAs exposure (37). The limit of detection for total arsenic and iAs (arsenite + arsenate), MMA, DMA, and arsenobetaine plus other arsenic cations was 0.1 $\mu\text{g/L}$. Because a major goal of the study was to evaluate the role of arsenic metabolism in diabetes development, we excluded participants with iAs (5.2%), MMA (0.8%), and DMA (0.03%) below the limit of detection from the original cohort.

From the risk assessment perspective, the sum of inorganic (iAs) and methylated (MMA, DMA) arsenic species in the urine was used to estimate arsenic exposure levels from multiple sources and exposure routes and can help to evaluate dose-response relationships related to exposure levels to inform risk assessment. The relative proportions of arsenic metabolites (iAs%, MMA%, and DMA%) were used to estimate the extent to which iAs is metabolized in the human body and inform on various arsenic metabolic profiles across individuals. This information is also useful as part of the susceptibility evaluation in risk assessment. The assessment of arsenic metabolism in addition to concentrations have been recommended by the 2013 National Research Council Report on arsenic (38).

Statistical Methods

We graphically described the distribution of arsenic metabolism in participants with and without diabetes using a triplot, a diagram with three axes that is well-suited to represent arsenic metabolism (Fig. 1).

The prospective associations between arsenic exposure and arsenic metabolism with incident diabetes were evaluated by Cox proportional hazards models. Arsenic exposure was evaluated based on the urinary concentration of the sum of inorganic and methylated arsenic species. We also evaluated the urinary concentration of iAs, MMA, and DMA in separate models. Arsenic metabolism was evaluated as iAs%, MMA%, and DMA%. Similar to previous studies (20,39,40), we first entered each arsenic metabolism biomarker alone in the regression model together with the sum of inorganic and methylated arsenic species to adjust for arsenic exposure. Entering each biomarker alone is difficult to interpret because the increase in iAs, for instance, could be related to a decrease in either MMA or DMA. To address this problem, we used a leave-one-out approach. In this method, two biomarkers are entered at a time, e.g., iAs% and MMA%, leaving out the third, i.e., DMA%, while holding constant urine arsenic concentrations. In the example, the regression coefficients for iAs% and MMA% estimate the hazard ratio associated with an increase in %iAs by decreasing DMA% and with an increase in MMA% by decreasing DMA%, respectively. This method has been used in the nutrition and hematology literature (41,42).

All arsenic variables were modeled per doubling or per 5% increment (in the log scale for urine arsenic species concentrations and in the original scale for % species) and using restricted cubic splines. The time scale for survival analysis was age. To handle left truncation induced at the time of enrollment and appropriately aligning risk sets on the age scale, the late-entry method was conducted using age at baseline as the individual entry time. The exit time for participants with newly diagnosed diabetes ($n = 218$) was the date of second or third visits. For participants with known diabetes status and data on diabetes duration, the exit date was the self-reported duration subtracted from the visit date ($n = 178$). To account for differences across geographical areas, we added the regions (Arizona, Oklahoma, and North and South Dakota) as strata to Cox proportional hazards models 1–4. This method allows the form of the underlying hazard function to vary across levels of study regions. Models were

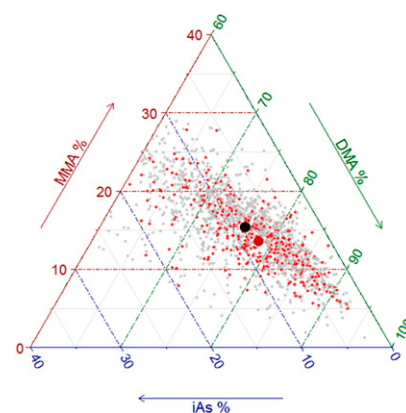


Figure 1—The triplot presents the distribution of arsenic metabolism biomarkers in participants with and without incident diabetes (red dots and gray dots, respectively). The large dark-red and black solid dots represent the compositional arsenic metabolism mean for participants with and without incident diabetes, respectively. iAs% is presented along the blue axis, MMA% along the red axis, and DMA% along the green axis. Compared with the black dot (participants without incident diabetes), the dark-red dot (participants with incident diabetes) was much closer to the apex of DMA% and farther away from the apex of MMA%, indicating that participants with incident diabetes had lower MMA% and higher DMA% at baseline.

adjusted progressively. Initially, we adjusted for sex and education (no high school, some high school, high school or more). We then adjusted further for smoking status (current, former, never) and alcohol drinking status (current, former, never). Finally, we further adjusted for BMI and waist-to-hip ratio as continuous variables. All models were adjusted for urine creatinine concentration to account for urine dilution (43). We confirmed that the proportional hazards assumption was fulfilled based on Schoenfeld residuals.

We conducted additional sensitivity analyses to evaluate the robustness of the primary findings. First, because the diabetes onset date is not exact, we conducted multiple logistic regression and Poisson regression to examine the robustness of the associations. The conclusions were consistent with our Cox proportional hazards model (not shown). Second, because mortality was high in the SHS population and a link exists between cancer and arsenic metabolism (44,45), we conducted a competing-risks analysis using death and cancer mortality as competing events, respectively, based on Fine

and Gray's (46) method, with similar results. We also used generalized γ -modeling to describe the competing relationship between mortality and incident diabetes, comparing the highest and the lowest quartiles of urine iAs concentrations (Supplementary Fig. 2) (47). Third, we applied two additional urine dilution correction methods by adjusting urine creatinine in the model and by adjusting specific gravity using Levine's approach, with consistent results (data not shown) (48). All statistical analyses were performed in Stata/IC 12 software (StataCorp, College Station, TX) and R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria [www.r-project.org]).

RESULTS

The median urine concentration of inorganic plus methylated arsenic species was 10.2 (interquartile range, 6.1–17.7) $\mu\text{g/L}$. Urine arsenic concentrations were higher in participants from Arizona (median 14.3 $\mu\text{g/L}$) followed by the Dakotas (11.9 $\mu\text{g/L}$) and Oklahoma (7.0 $\mu\text{g/L}$). The median (interquartile range) for iAs%, MMA%, and DMA% was 8.3% (5.7–11.3%), 15.2% (11.7–18.8%), and 76.4% (70.3–81.4%), respectively. Men, participants from the Dakotas, current smokers, and participants with lower BMI had higher MMA% and, correspondingly, lower DMA% (Supplementary Fig. 3).

Over 11,263.2 person-years of follow-up, 396 participants developed diabetes. Diabetes incidence was 35.2 per 1,000 person-years. Participants with incident diabetes were more likely to be female, from Arizona, and obese at baseline (Table 1). Younger age was borderline associated with incident diabetes ($P = 0.05$). Urine concentrations of inorganic plus methylated arsenic species were similar in participants with and without incident diabetes. Participants with incident diabetes had lower MMA% and higher DMA% than those without incident diabetes (Table 1 and Fig. 1). Arsenic exposure, assessed as the summed concentrations of inorganic and methylated arsenic species or as each of the individual arsenic species in urine, was not associated with incident diabetes in any of the multivariable adjusted models (Table 2 and Supplementary Fig. 4).

For arsenic metabolism, the multi-adjusted hazard ratio (95% CI) of diabetes incidence per 5% increase

Table 1—Characteristics of SHS participants free of diabetes at baseline (1989–1991)

	No DM events (<i>n</i> = 1,298, 76.6%)	DM events (<i>n</i> = 396, 23.4%)	<i>P</i> value
Age (years)	54.6 (48.8–61.8)	53.3 (48.5–60.3)	0.05
Male sex	610 (47.0)	153 (38.6)	<0.01
Location			<0.01
Arizona	255 (19.7)	114 (28.8)	
Oklahoma	504 (38.8)	109 (27.5)	—
North and South Dakota	539 (41.5)	173 (43.7)	—
Education			0.06
No high school	230 (17.7)	91 (23.0)	—
Some high school	305 (23.5)	89 (22.5)	—
High school or more	763 (58.8)	216 (54.6)	—
Smoking			0.05
Never	353 (27.2)	126 (31.8)	—
Former	398 (30.7)	129 (32.6)	—
Current	547 (42.1)	141 (35.6)	—
Alcohol			0.19
Never	158 (12.2)	61 (15.4)	—
Former	499 (38.4)	154 (38.9)	—
Current	641 (49.4)	181 (45.7)	—
BMI (kg/m^2)	28.0 (25.0–31.9)	30.9 (28.1–35.3)	<0.01
Waist-to-hip ratio	0.94 (0.89–0.98)	0.96 (0.92–0.99)	<0.01
Waist circumference (cm)	98 (91–107)	106 (98–116)	<0.01
Body fat (%)	33.3 (27.1–40.8)	38.5 (31.1–44.3)	<0.01
Urine creatinine (g/L)	1.3 (0.8–1.8)	1.2 (0.9–1.7)	0.80
eGFR (mL/min/1.73 m^2)	81.3 (71.6–92.7)	81.1 (70.8–93.7)	0.48
Fasting glucose (mg/dL)	100 (93–107)	106 (98–113)	<0.01
HbA _{1c} (%)	5.0 (4.7–5.4) ^a	5.3 (4.9–5.7) ^b	<0.01
HbA _{1c} (mmol/mol)	31.2 (27.9–35.5) ^a	34.4 (30.1–38.6) ^b	<0.01
Arsenic exposure			
iAs + methylated arsenic ($\mu\text{g/g}$)	8.7 (5.3–13.8)	9.1 (5.9–14.0)	0.32
iAs ($\mu\text{g/g}$)	0.7 (0.4–1.4)	0.7 (0.4–1.3)	0.87
MMA ($\mu\text{g/g}$)	1.3 (0.8–2.2)	1.2 (0.8–2.1)	0.58
DMA ($\mu\text{g/g}$)	6.4 (4.0–10.3)	7.0 (4.4–11.2)	0.16
Arsenic metabolism			
iAs%	8.4 (5.7–11.6)	8.1 (5.7–10.7)	0.09
MMA%	15.5 (12.0–19.1)	14.0 (11.2–17.1)	<0.01
DMA%	75.9 (69.6–81.3)	77.4 (72.6–81.9)	<0.01

Data are median (interquartile range) or *n* (%). DM, diabetes mellitus; eGFR, estimated glomerular filtration rate. ^a*n* = 1,214. ^b*n* = 375.

in arsenic metabolism biomarkers entered one by one in the model (conventional approach) was 1.00 (0.89–1.12) for iAs%, 0.84 (0.76–0.94) for MMA%, and 1.07 (1.00–1.15) for DMA% (Table 3, model 4). Using the leave-one-out approach, we confirmed that higher MMA% was associated with lower diabetes incidence. The hazard ratios (95% CI) of diabetes incidence for a 5% increase in MMA% were 0.77 (0.63–0.93) and 0.82 (0.73–0.92) when iAs% and DMA%, respectively, were left out of the model (Table 3, model 4). In other words, when MMA% and iAs% were in the model, the hazard ratio of incident

diabetes of MMA% was the effect of replacing DMA% with MMA% while holding iAs% constant. The same interpretation applied when DMA% and iAs% and when MMA% and DMA% were in the model simultaneously. Consistently, higher MMA% was related to lower diabetes incidence, showing a linear relationship in flexible dose-response analyses when either iAs% or DMA% were left out of the model (Fig. 2). DMA% was associated with higher diabetes incidence only when substituted for MMA%, and iAs% was associated with higher diabetes incidence only when substituted for MMA% (Table 3 and Fig. 2). The

Table 2—Incident diabetes per doubling increase in urine concentrations of iAs, MMA, DMA and the sum of iAs, MMA, and DMA ($\mu\text{g/g}$ creatinine)

Arsenic (per doubling increase)	Model 1	Model 2	Model 3	Model 4
iAs	0.93 (0.86–1.01)	0.93 (0.85–1.01)	0.94 (0.86–1.02)	0.98 (0.90–1.06)
MMA	0.85 (0.76–0.94)	0.83 (0.75–0.92)	0.84 (0.75–0.93)	0.90 (0.81–1.00)
DMA	1.00 (0.90–1.12)	0.96 (0.86–1.08)	0.97 (0.87–1.09)	0.98 (0.87–1.11)
iAs + methylated arsenic species	0.95 (0.85–1.07)	0.92 (0.82–1.03)	0.93 (0.83–1.05)	0.96 (0.85–1.08)

Data are hazard ratio (95% CI). Model 1: stratified by study center and adjusted for age (age as time metric and age at baseline were treated as staggered entries). Model 2: further adjusted for sex and education. Model 3: further adjusted for smoking and alcohol drinking. Model 4: further adjusted for BMI and waist-to-hip ratio. Urine creatinine at baseline was not associated with incident diabetes (the hazard ratio of incident diabetes per mg/dL increase in urine creatinine was 1.05 [0.92–1.19]).

association between MMA% and diabetes incidence was similar by age, sex, study site, obesity, and the sum of inorganic and methylated arsenic species concentrations (Supplementary Table 1).

CONCLUSIONS

Arsenic metabolism, but not iAs exposure, was prospectively associated with diabetes incidence in American Indians from Arizona, Oklahoma, and North and South Dakota. Higher iAs% and DMA% in urine, due to lower MMA%, was associated with higher diabetes incidence. Consistently, higher MMA% was associated with lower risk of diabetes. The associations persisted after adjustment for sociodemographic factors, smoking, alcohol, kidney function, and measures of adiposity. These novel findings

support that arsenic metabolism patterns, in particular lower MMA%, may be a predisposing factor for diabetes. Arsenic exposure, measured by the concentration of inorganic plus methylated arsenic species in urine, however, was not associated with diabetes incidence in this study population. The study was conducted in a population with a high burden of obesity and diabetes (49) and characterized by low to moderate arsenic exposure levels.

Nongenetic determinants of arsenic metabolism include sex (women have higher DMA% than men), smoking (never smokers generally have higher DMA% than current smokers), nutritional status (dietary folate and vitamin deficiencies are associated with lower DMA%), and BMI (obese individuals have higher DMA%) (9). In women,

MMA% decreases and DMA% increases during pregnancy (50,51). Although the risk of gestational diabetes is also increased, a connection with changes in arsenic metabolic patterns during pregnancy is unknown (52,53). Of note, the present study shows that the arsenic metabolic pattern associated with increased diabetes risk parallels that observed during pregnancy (i.e., lower MMA%, higher DMA%). Genetic determinants, especially variants in arsenic (III) methyltransferase (*AS3MT*), have also been related to arsenic methylation patterns in urine (54,55). Additional research is needed to evaluate whether genetic variants play a role in the connection between arsenic metabolism profile and diabetes.

Little is known about arsenic metabolism in diabetes compared with its role in cancer and cardiovascular disease

Table 3—Incident diabetes per 5% increase in arsenic metabolism biomarkers iAs%, MMA%, and DMA%

Arsenic metabolism	Model 1	Model 2	Model 3	Model 4
Conventional approach (per 5% increase)				
iAs%	0.89 (0.80–1.00)	0.92 (0.82–1.03)	0.93 (0.83–1.05)	1.00 (0.89–1.12)
MMA%	0.77 (0.69–0.85)	0.78 (0.70–0.86)	0.78 (0.70–0.87)	0.84 (0.76–0.94)
DMA%	1.14 (1.07–1.22)	1.14 (1.06–1.21)	1.13 (1.06–1.21)	1.07 (1.00–1.15)
Leave-one-out approach				
iAs% (per 5% increase) corresponds to:				
MMA% (per 5% decrease)	1.32 (1.10–1.59)	1.34 (1.11–1.61)	1.35 (1.12–1.62)	1.31 (1.08–1.58)
DMA% (per 5% decrease)	1.01 (0.90–1.13)	1.03 (0.92–1.15)	1.04 (0.92–1.16)	1.08 (0.96–1.21)
MMA% (per 5% increase) corresponds to:				
iAs% (per 5% decrease)	0.76 (0.63–0.91)	0.75 (0.62–0.90)	0.74 (0.62–0.89)	0.77 (0.63–0.93)
DMA% (per 5% decrease)	0.77 (0.69–0.86)	0.77 (0.69–0.86)	0.77 (0.69–0.86)	0.82 (0.73–0.92)
DMA% (per 5% increase) corresponds to:				
iAs% (per 5% decrease)	0.99 (0.88–1.11)	0.97 (0.87–1.09)	0.96 (0.86–1.08)	0.93 (0.82–1.05)
MMA% (per 5% decrease)	1.31 (1.17–1.46)	1.30 (1.16–1.45)	1.30 (1.16–1.45)	1.21 (1.08–1.36)

Data are hazard ratio (95% CI). Because the three biomarkers equal 100%, models entered two biomarkers at a time. All models were adjusted for the sum of iAs, MMA, and DMA ($\mu\text{g/g}$ creatinine). In the conventional approach, each arsenic metabolism biomarker (iAs%, MMA%, and DMA%) is entered alone in the model. In the leave-one-out approach, two arsenic metabolism biomarkers are entered in the model. In that model, an increase in each arsenic metabolism biomarker corresponds to a decrease in the biomarker that is left out of the model. For instance, an increase in iAs% corresponds to a decrease in MMA% when DMA% is included in the model and MMA% is left out. The magnitude of the association for iAs% when MMA% is left out will be the same but in the opposite direction of MMA% when iAs% is left out. Both in the conventional approach and in the leave-one-out approach we adjusted for the sum of inorganic and methylated arsenic concentrations in urine to hold arsenic exposure levels constant. Model 1: stratified by study center, adjusted for age (age as time metric and age at baseline were treated as staggered entries), and adjusted for the sum of iAs and methylated arsenic concentrations. Model 2: further adjusted for sex and education. Model 3: further adjusted for smoking and alcohol drinking. Model 4: further adjusted for BMI and waist-to-hip ratio.

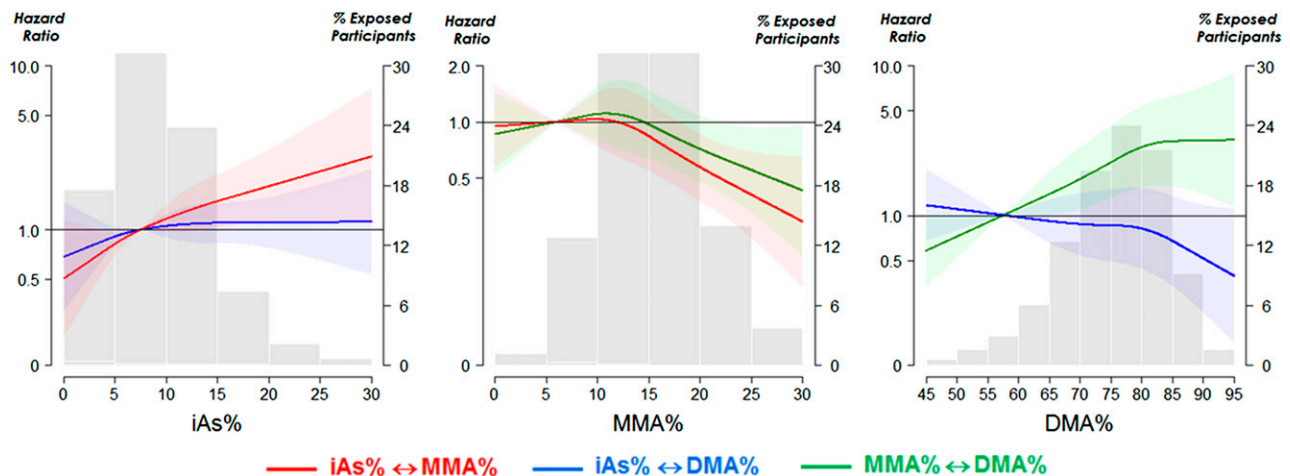


Figure 2—Hazard ratios for incident diabetes by arsenic metabolism biomarkers. Solid lines (shaded area) represent adjusted hazard ratios (95% CI) based on restricted quadratic splines with knots at the 10th, 50th, and 90th percentiles. The reference value was set at the 10th percentile of each arsenic metabolism biomarker. The solid line represents the hazard ratio for iAs% when it replaces MMA% (red line) and DMA% (blue line) in the left panel, the hazard ratio for MMA% when it replaces iAs% (red line) and DMA% (green line) in the middle panel, and the hazard ratio for DMA% when it replaces iAs% (blue line) and MMA% (green line) in the right panel. The histogram represents the distributions of arsenic metabolism biomarkers (iAs%, MMA%, and DMA%) among the study participants. The extreme tails of the histogram were truncated because 12 participants had an iAs% >30%, 25 had an MMA% >30%, 10 had a DMA% <45%, and 1 had a DMA% >95%.

(11,14,56–58). In studies conducted mostly in Taiwan and Bangladesh, higher MMA% was associated with the development of lung (57), bladder (11), and skin (56) cancers and with cardiovascular disease, including atherosclerosis and peripheral vascular disease (14,58). In one small case-control study from Bangladesh, higher DMA% was associated with increased prevalence of diabetes, although the association was not statistically significant (20). High BMI has also been significantly associated with low MMA% and high DMA% in urine in adults from Mexico and the SHS (21,22). In the present study, adjusting for baseline BMI and waist-to-hip ratio slightly attenuated the association between arsenic metabolism and incident diabetes, although the association remained. How this specific pattern (low MMA% with either high iAs% or high DMA%) may affect individual susceptibility to endocrine and metabolic diseases remains unclear.

Substantial experimental research supports the role of arsenic exposure in diabetes development (2,3). Experimental studies in general have not focused on differences by arsenic metabolism. High MMA% may be considered a marker of insufficient methylation capacity to DMA. Experimental studies have shown that methylation could be a bioactivation process, with DMA(III) being a potent and highly

toxic dimethylated arsenic species (59,60). In adipocytes, DMA(III) impairs insulin-stimulated glucose uptake (16). In addition, DMA(III) may induce pancreatic β -cell apoptosis and inhibit glucose-stimulated insulin secretion in murine pancreatic islet cells (61,62). These experimental findings were consistent with a cross-sectional study from Mexico, where the concentrations of DMA(III) in urine were associated with diabetes prevalence (5). In the present study, similar to other large epidemiologic studies, we measured total MMA and DMA because MMA(III) and DMA(III) are unstable in urine and quickly oxidize to their pentavalent forms (63). The rapid oxidation of MMA(III) and DMA(III) in urine to their pentavalent counterparts makes estimation of the trivalent methylated species very difficult in epidemiological studies. However, participants with higher DMA% may be exposed to more DMA(III) given the same amount of total iAs exposure.

The association of arsenic metabolism with diabetes could also be related to one-carbon metabolism because *S*-adenosylmethionine (SAM) is the methyl donor for arsenic metabolism (24,64). Experimental evidence has shown that SAM plays an important role in lipogenesis and in the development of diabetes (26,65,66). An *in vitro* study in *Caenorhabditis elegans*, an experimental model for human diseases

and metabolic pathways (67,68), found that the synthesis of SAM regulated the expression of genes required for adequate lipid metabolism (65). In *HepG2* human hepatocytes, the optimal balance between SAM and *S*-adenosylhomocysteine is critical to maintain appropriate expression of gluconeogenic enzymes (66). In addition, in a cross-sectional study of 50–75-year-old adults from the Netherlands ($n = 648$), plasma SAM was positively associated with fat mass and truncal adiposity, although reverse causation could not be excluded (69). We cannot discount the possibility that arsenic metabolism acts as a marker of one-carbon metabolism. In the present study, we had no serological measures of one-carbon metabolism, and dietary estimations were only available in 20% of the sample. In any case, the findings indicate that more research is needed to understand the impact of arsenic methylation and other methylation processes related to one-carbon metabolism on the development of diabetes.

We found no association between arsenic exposure and incident diabetes, although cross-sectionally, we had found an association (6). iAs and its methylated metabolites may induce diabetes by impairing insulin production by pancreatic β -cells or inhibiting basal or insulin-stimulated glucose uptake by peripheral tissues (10,70). Relevant

mechanisms by which arsenic could affect β -cell function and insulin sensitivity include oxidative stress, glucose uptake and transport, gluconeogenesis, adipocyte differentiation, calcium signaling, and epigenetic effects (2,10). A number of studies have reported a prospective association between arsenic exposure and diabetes development (3,7,8). It is possible that arsenic exposure is not a risk factor for diabetes in the current study population. At the same time, the presence of an association between arsenic exposure and diabetes cross-sectionally, but not prospectively, could be related to competing risk of premature death and differential survival bias that may mask the true association in the present study. Because arsenic was strongly associated with diabetes at baseline and the prevalence of diabetes at baseline was 50% (6), another possible explanation for the lack of association is that the pool of susceptible participants was too small for the association to be seen prospectively. In support of this possibility, age was not positively associated with diabetes incidence either (Table 1). BMI, however, remained a strong risk factor.

Strengths of this study include the standardized protocol to collect data over the follow-up period, high-quality laboratory methods for measuring concentrations of urine arsenic species at baseline, and careful modeling of the dynamic of arsenic metabolism, including the leave-one-out approach. Another advantage is that we used the sum of iAs and methylated arsenic metabolites in the urine to represent an iAs exposure that integrates various sources and routes of exposure and avoids the measurement error problems associated with seafood exposure. This study also had several limitations. First, the population was between 40 and 74 years of age, and the burden of diabetes at baseline was already 50%. Thus, participants susceptible to developing diabetes at baseline were possibly different from the source population. Studies in younger populations with a lower prevalence of diabetes at baseline are needed. Second, the study was observational, and we cannot exclude the possibility of residual or unmeasured confounding by, for example, accurate smoking and drinking information or other measures

of one-carbon metabolism. However, the results were consistent after further adjustment for folic acid, vitamin B6, and cobalamin based on food frequency questionnaire in the 20% subsample with this information available (data not shown).

In conclusion, arsenic metabolism, in particular low MMA%, is associated with increased incidence of diabetes and could reflect individual susceptibility for diabetes development. Arsenic metabolism is related to one-carbon metabolism and could be functioning as a surrogate measure of one-carbon metabolism. Research is needed to assess the relationship between arsenic metabolism and diabetes in various populations, evaluate the diabetogenic role of arsenic metabolism in experimental settings, and clarify whether the development of diabetes is related to arsenic metabolism specifically or to one-carbon metabolism in general.

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