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

Chin-Chi Kuo,[1–4] Barbara V. Howard,[5,6] Jason G. Umans,[5,6] Matthew O. Gribble,[7] Lyle G. Best,[8] Kevin A. Francesconi,[9] Walter Goessler,[9] Elisa Lee,[10] Eliseo Guallar,[1,3,11] and Ana Navas-Acien[1–3,12]

Diabetes Care 2015;38:620–627 | DOI: 10.2337/dc14-1641

EPIDEMIOLOGY/HEALTH SERVICES RESEARCH

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 antidiabetic 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 μg/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

[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
[2]Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
[3]Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Medical Institutions, Baltimore, MD
[4]Kidney Institute and Division of Nephrology, Department of Internal Medicine, China Medical University Hospital and College of Medicine, China Medical University, Taichung, Taiwan
[5]MedStar Health Research Institute, Hyattsville, MD
[6]Georgetown-Howard Universities Center for Clinical and Translational Science, Washington, DC
[7]University of Southern California, Los Angeles, CA
[8]Missouri Breaks Industries Research, Inc., Timber Lake, SD
[9]Institute of Chemistry – Analytical Chemistry, Karl-Franzens University Graz, Graz, Austria
[10]Center for American Indian Health Research, College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, OK
[11]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD
[12]Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD

Corresponding author: Chin-Chi Kuo, chinchik@gmail.com.

Received 5 July 2014 and accepted 11 December 2014.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc14-1641/-/DC1.

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 $\leq -70^{\circ}C$ 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 $\geq$126 mg/dL, plasma glucose $\geq$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] μg/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 μg/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 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.

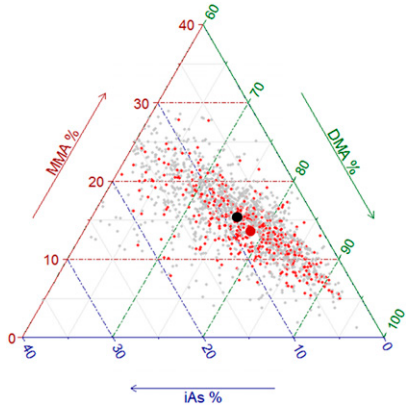

Figure 1—The triplot presents the distribution of arsenic metabolism biomarkers in participants with and without incident diabetes (red dots and gray dots, respectively). The large dark-red and black solid dots represent the compositional arsenic metabolism mean for participants with and without incident 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.

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) μg/L. Urine arsenic concentrations were higher in participants from Arizona (median 14.3 μg/L) followed by the Dakotas (11.9 μg/L) and Oklahoma (7.0 μg/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 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 1—Characteristics of SHS participants free of diabetes at baseline (1989–1991)

	No DM events (n = 1,298, 76.6%)	DM events (n = 396, 23.4%)	P 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 (μg/g)	8.7 (5.3–13.8)	9.1 (5.9–14.0)	0.32
iAs (μg/g)	0.7 (0.4–1.4)	0.7 (0.4–1.3)	0.87
MMA (μg/g)	1.3 (0.8–2.2)	1.2 (0.8–2.1)	0.58
DMA (μg/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.

Table 2—Incident diabetes per doubling increase in urine concentrations of iAs, MMA, DMA and the sum of iAs, MMA, and DMA (μg/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 (μg/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.

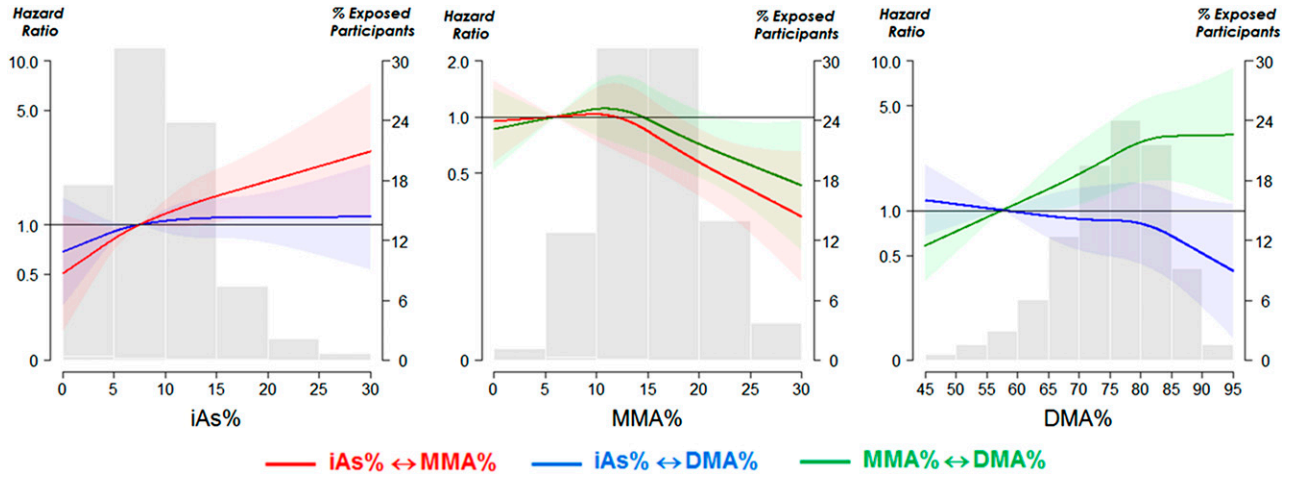

Figure 2—Hazard ratios for incident diabetes by arsenic metabolism biomarkers. Solid lines (shaded area) represent adjusted hazard ratios (95% CI) based on restricted quadratic splines with knots at the 10th, 50th, and 90th percentiles. The reference value was set at the 10th percentile of each arsenic 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.

Funding. This study was supported by grants from the National Heart, Lung, and Blood Institute (R01-HL-090863, HL-41642, HL-41652, HL-41654, and HL-65521) and the National Institute of Environmental Health Sciences (R01-ES-021367 and P30-ES-03819).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. C.-C.K., M.O.G., E.G., and A.N.-A. contributed to the preparation of the research data, statistical analysis, and writing of the manuscript. B.V.H., J.G.U., L.G.B., and E.L. contributed as the primary investigators of the SHS and to the preparation of the research data. K.A.F. and W.G. contributed to the arsenic measurements in the SHS participants. A.N.-A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in abstract form at the 2014 Conference of the International Society for Environmental Epidemiology, Seattle, WA, 24–28 August 2014.

References

1. World Health Organization. *Exposure to Arsenic: A Major Public Health Concern*. Geneva, Switzerland, World Health Organization, 2010
2. Maull EA, Ahsan H, Edwards J, et al. Evaluation of the association between arsenic and diabetes: a National Toxicology Program workshop review. Environ Health Perspect 2012;120:1658–1670
3. Kuo CC, Moon K, Thayer KA, Navas-Acien A. Environmental chemicals and type 2 diabetes: an updated systematic review of the epidemiologic evidence. Curr Diab Rep 2013;13:831–849
4. Navas-Acien A, Silbergeld EK, Streeter RA, Clark JM, Burke TA, Guallar E. Arsenic exposure and type 2 diabetes: a systematic review of the experimental and epidemiological evidence. Environ Health Perspect 2006;114:641–648
5. Del Razo LM, García-Vargas GG, Valenzuela OL, et al. Exposure to arsenic in drinking water is associated with increased prevalence of diabetes: a cross-sectional study in the Zimapán and Lagunera regions in Mexico. Environ Health 2011;10:73
6. Gribble MO, Howard BV, Umans JG, et al. Arsenic exposure, diabetes prevalence, and diabetes control in the Strong Heart Study. Am J Epidemiol 2012;176:865–874
7. James KA, Marshall JA, Hokanson JE, Meliker JR, Zerbe GO, Byers TE. A case-cohort study examining lifetime exposure to inorganic arsenic in drinking water and diabetes mellitus. Environ Res 2013;123:33–38
8. Kim NH, Mason CC, Nelson RG, et al. Arsenic exposure and incidence of type 2 diabetes in Southwestern American Indians. Am J Epidemiol 2013;177:962–969
9. Tseng CH. A review on environmental factors regulating arsenic methylation in humans. Toxicol Appl Pharmacol 2009;235:338–350
10. National Research Council. *Critical Aspects of EPA's IRIS Assessment of Inorganic Arsenic: Interim Report*. Washington, DC, The National Academies Press, 2013
11. Chen Y-C, Su H-JJ, Guo Y-LL, et al. Arsenic methylation and bladder cancer risk in Taiwan. Cancer Causes Control 2003;14:303–310
12. Steinmaus C, Bates MN, Yuan Y, et al. Arsenic methylation and bladder cancer risk in case-control studies in Argentina and the United States. J Occup Environ Med 2006;48:478–488
13. Yu RC, Hsu KH, Chen CJ, Froines JR. Arsenic methylation capacity and skin cancer. Cancer Epidemiol Biomarkers Prev 2000;9:1259–1262
14. Huang YL, Hsueh YM, Huang YK, Yip PK, Yang MH, Chen CJ. Urinary arsenic methylation capability and carotid atherosclerosis risk in subjects living in arsenicosis-hyperendemic areas in southwestern Taiwan. Sci Total Environ 2009;407:2608–2614
15. Wu M-M, Chiou H-Y, Hsueh Y-M, et al. Effect of plasma homocysteine level and urinary monomethylarsonic acid on the risk of arsenic-associated carotid atherosclerosis. Toxicol Appl Pharmacol 2006;216:168–175
16. Walton FS, Harmon AW, Paul DS, Drobná Z, Patel YM, Styblo M. Inhibition of insulin-dependent glucose uptake by trivalent arsenicals: possible mechanism of arsenic-induced diabetes. Toxicol Appl Pharmacol 2004;198:424–433
17. Paul DS, Harmon AW, Devesa V, Thomas DJ, Stýblo M. Molecular mechanisms of the diabetogenic effects of arsenic: inhibition of insulin signaling by arsenite and methylarsonous acid. Environ Health Perspect 2007;115:734–742
18. Vahter M. Mechanisms of arsenic biotransformation. Toxicology 2002;181-182:211–217
19. Petrick JS, Ayala-Fierro F, Cullen WR, Carter DE, Vasken Aposhian H. Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. Toxicol Appl Pharmacol 2000;163:203–207
20. Nizam S, Kato M, Yatsuya H, et al. Differences in urinary arsenic metabolites between diabetic and non-diabetic subjects in Bangladesh. Int J Environ Res Public Health 2013;10:1006–1019
21. Gomez-Rubio P, Roberge J, Arendell L, et al. Association between body mass index and

arsenic methylation efficiency in adult women from southwest U.S. and northwest Mexico. Toxicol Appl Pharmacol 2011;252:176–182
22. Gribble MO, Crainiceanu CM, Howard BV, et al. Body composition and arsenic metabolism: a cross-sectional analysis in the Strong Heart Study. Environ Health 2013;12:107
23. Hall MN, Gamble MV. Nutritional manipulation of one-carbon metabolism: effects on arsenic methylation and toxicity. J Toxicol 2012; 2012:595307
24. Locasale JW. Serine, glycine and one-carbon units: cancer metabolism in full circle. Nat Rev Cancer 2013;13:572–583
25. Baccarelli A, Rienstra M, Benjamin EJ. Cardiovascular epigenetics: basic concepts and results from animal and human studies. Circ Cardiovasc Genet 2010;3:567–573
26. Ngo S, Li X, O'Neill R, Bhoothpur C, Gluckman P, Sheppard A. Elevated *S*-adenosylhomocysteine alters adipocyte functionality with corresponding changes in gene expression and associated epigenetic marks. Diabetes 2014;63:2273–2283
27. Krishnaveni GV, Veena SR, Karat SC, Yajnik CS, Fall CH. Association between maternal folate concentrations during pregnancy and insulin resistance in Indian children. Diabetologia 2014;57:110–121
28. Howard BV, Welty TK, Fabsitz RR, et al. Risk factors for coronary heart disease in diabetic and nondiabetic Native Americans. The Strong Heart Study. Diabetes 1992;41(Suppl. 2):4–11
29. Navas-Acien A, Umans JG, Howard BV, et al. Urine arsenic concentrations and species excretion patterns in American Indian communities over a 10-year period: the Strong Heart Study. Environ Health Perspect 2009;117:1428–1433
30. Lee ET, Howard BV, Wang W, et al. Prediction of coronary heart disease in a population with high prevalence of diabetes and albuminuria: the Strong Heart Study. Circulation 2006; 113:2897–2905
31. Lee ET, Welty TK, Fabsitz R, et al. The Strong Heart Study. A study of cardiovascular disease in American Indians: design and methods. Am J Epidemiol 1990;132:1141–1155
32. Stoddart ML, Jarvis B, Blake B, et al. Recruitment of American Indians in epidemiologic research: the Strong Heart Study. Am Indian Alsk Native Ment Health Res 2000;9:20–37
33. Howard BV, Lee ET, Fabsitz RR, et al. Diabetes and coronary heart disease in American Indians: the Strong Heart Study. Diabetes 1996; 45(Suppl. 3):S6–S13
34. Levey AS, Stevens LA, Schmid CH, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150:604–612
35. Scheer J, Findenig S, Goessler W, et al. Arsenic species and selected metals in human urine: validation of HPLC/ICPMS and ICPMS procedures for a long-term population-based epidemiological study. Anal Methods 2012;4:406–413
36. National Research Council. *Arsenic in Drinking Water*. Washington, DC, National Academies Press, 1999
37. Navas-Acien A, Francesconi KA, Silbergeld EK, Guallar E. Seafood intake and urine concentrations of total arsenic, dimethylarsinate and arsenobetaine in the US population. Environ Res 2011;111:110–118
38. Committee on Inorganic Arsenic; Board on Environmental Studies and Toxicology; Division of Earth and Life Studies; National Research Council. *Critical Aspects of EPA's IRIS Assessment of Inorganic Arsenic: Interim Report (2014)*. Washington, DC, National Academies Press, 2014
39. Gilbert-Diamond D, Li Z, Perry AE, Spencer SK, Gandolfi AJ, Karagas MR. A population-based case-control study of urinary arsenic species and squamous cell carcinoma in New Hampshire, USA. Environ Health Perspect 2013;121:1154–1160
40. Lindberg AL, Rahman M, Persson LA, Vahter M. The risk of arsenic induced skin lesions in Bangladeshi men and women is affected by arsenic metabolism and the age at first exposure. Toxicol Appl Pharmacol 2008;230:9–16
41. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65(4 Suppl.):1220S–1228S; discussion 1229S–1231S
42. Donahue JG, Nelson KE, Muñoz A, et al. Transmission of HIV by transfusion of screened blood. N Engl J Med 1990;323:1709
43. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 2005;113:192–200
44. Nwaneri C, Cooper H, Bowen-Jones D. Mortality in type 2 diabetes mellitus: magnitude of the evidence from a systematic review and meta-analysis. Brit J Diabetes Vasc Dis 2013; 13:192–207
45. Martinez VD, Vucic EA, Adonis M, Gil L, Lam WL. Arsenic biotransformation as a cancer promoting factor by inducing DNA damage and disruption of repair mechanisms. Mol Biol Int 2011;2011:718974
46. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496–509
47. Cox C, Chu H, Schneider MF, Muñoz A. Parametric survival analysis and taxonomy of hazard functions for the generalized gamma distribution. Stat Med 2007;26:4352–4374
48. Levine L, Fahy J. Evaluation of urinary lead determination: I. The significance of the specific gravity. J Ind Hyg Toxicol 1945;27:217–223
49. Thompson FC. *Vital Statistics of the United States 2012: Births, Life Expectancy, Deaths, and Selected Health Data*. 5th ed. Blue Ridge Summit, PA, Bernan Press, 2012
50. Hopenhayn C, Huang B, Christian J, et al. Profile of urinary arsenic metabolites during pregnancy. Environ Health Perspect 2003;111: 1888–1891
51. Gardner RM, Engström K, Bottai M, et al. Pregnancy and the methyltransferase genotype independently influence the arsenic methylation phenotype. Pharmacogenet Genomics 2012;22:508–516
52. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am J Clin Nutr 2000; 71(Suppl.):1256S–1261S
53. Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. Diabetes Care 2007;30(Suppl. 2):S112–S119
54. Pierce BL, Kibriya MG, Tong L, et al. Genome-wide association study identifies chromosome 10q24.32 variants associated with arsenic metabolism and toxicity phenotypes in Bangladesh. PLoS Genet 2012;8:e1002522
55. Tellez-Plaza M, Gribble MO, Voruganti VS, et al. Heritability and preliminary genome-wide linkage analysis of arsenic metabolites in urine. Environ Health Perspect 2013;121:345–351
56. Yu RC, Hsu KH, Chen CJ, Froines JR. Arsenic methylation capacity and skin cancer. Cancer Epidemiol Biomarkers Prev 2000;9:1259–1262
57. Steinmaus C, Yuan Y, Kalman D, et al. Individual differences in arsenic metabolism and lung cancer in a case-control study in Cordoba, Argentina. Toxicol Appl Pharmacol 2010;247:138–145
58. Tseng CH, Huang YK, Huang YL, et al. Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in Blackfoot disease-hyperendemic villages in Taiwan. Toxicol Appl Pharmacol 2005;206:299–308
59. Mass MJ, Tennant A, Roop BC, et al. Methylated trivalent arsenic species are genotoxic. Chem Res Toxicol 2001;14:355–361
60. Agusa T, Fujihara J, Takeshita H, Iwata H. Individual variations in inorganic arsenic metabolism associated with AS3MT genetic polymorphisms. Int J Mol Sci 2011;12:2351–2382
61. Douillet C, Currier J, Saunders J, Bodnar WM, Matoušek T, Stýblo M. Methylated trivalent arsenicals are potent inhibitors of glucose stimulated insulin secretion by murine pancreatic islets. Toxicol Appl Pharmacol 2013;267:11–15
62. Lu TH, Su CC, Chen YW, et al. Arsenic induces pancreatic β-cell apoptosis via the oxidative stress-regulated mitochondria-dependent and endoplasmic reticulum stress-triggered signaling pathways. Toxicol Lett 2011;201:15–26
63. Kalman DA, Dills RL, Steinmaus C, et al. Occurrence of trivalent monomethyl arsenic and other urinary arsenic species in a highly exposed juvenile population in Bangladesh. J Expo Sci Environ Epidemiol 2014;24:113–120
64. Vahter ME. Interactions between arsenic-induced toxicity and nutrition in early life. J Nutr 2007;137:2798–2804
65. Walker AK, Jacobs RL, Watts JL, et al. A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. Cell 2011;147:840–852
66. Jackson MI, Cao J, Zeng H, Uthus E, Combs GF Jr. *S*-adenosylmethionine-dependent protein methylation is required for expression of selenoprotein P and gluconeogenic enzymes in HepG2 human hepatocytes. J Biol Chem 2012;287:36455–36464
67. Leung MC, Williams PL, Benedetto A, et al. *Caenorhabditis elegans*: an emerging model in biomedical and environmental toxicology. Toxicol Sci 2008;106:5–28
68. Hashmi S, Wang Y, Parhar RS, et al. A *C. elegans* model to study human metabolic regulation. Nutr Metab (Lond) 2013;10:31
69. Elshorbagy AK, Nijpels G, Valdivia-Garcia M, et al. *S*-adenosylmethionine is associated with fat mass and truncal adiposity in older adults. J Nutr 2013;143:1982–1988
70. Paul DS, Hernández-Zavala A, Walton FS, et al. Examination of the effects of arsenic on glucose homeostasis in cell culture and animal studies: development of a mouse model for arsenic-induced diabetes. Toxicol Appl Pharmacol 2007;222:305–314