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Association of Low-Moderate Arsenic Exposure and Arsenic Metabolism with Incident Diabetes and Insulin Resistance in the Strong Heart Family Study

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Background: High arsenic exposure has been related to diabetes, but at low-moderate levels the evidence is mixed. Arsenic metabolism, which is partly genetically controlled and may rely on certain B vitamins, plays a role in arsenic toxicity.

Objective: We evaluated the prospective association of arsenic exposure and metabolism with type 2 diabetes and insulin resistance.

Methods: We included 1,838 American Indian men and women free of diabetes (median age, 36 y). Arsenic exposure was assessed as the sum of inorganic arsenic (iAs), monomethylarsonate (MMA), and dimethylarsinate (DMA) urine concentrations (ΣAs). Arsenic metabolism was evaluated by the proportions of iAs, MMA, and DMA over their sum (iAs%, MMA%, and DMA%). Homeostasis model assessment for insulin resistance (HOMA2-IR) was measured at baseline and follow-up visits. Incident diabetes was evaluated at follow-up.

Results: Median ΣAs, iAs%, MMA%, and DMA% was 4.4 μg/g creatinine, 9.5%, 14.4%, and 75.6%, respectively. Over 10,327 person-years of follow-up, 252 participants developed diabetes. Median HOMA2-IR at baseline was 1.5. The fully adjusted hazard ratio [95% confidence interval (CI)] for incident diabetes per an interquartile range increase in ΣAs was 1.57 (95% CI: 1.18, 2.08) in participants without prediabetes at baseline. Arsenic metabolism was not associated with incident diabetes. ΣAs was positively associated with HOMA2-IR at baseline but negatively with HOMA2-IR at follow-up. Increased MMA% was associated with lower HOMA2-IR when either iAs% or DMA% decreased. The association of arsenic metabolism with HOMA2-IR differed by B-vitamin intake and *AS3MT* genetics variants.

Conclusions: Among participants without baseline prediabetes, arsenic exposure was associated with incident diabetes. Low MMA% was cross-sectional and prospectively associated with higher HOMA2-IR. Research is needed to confirm possible interactions of arsenic metabolism with B vitamins and *AS3MT* variants on diabetes risk. https://doi.org/10.1289/EHP2566

Introduction

Inorganic arsenic (iAs) is a toxicant and carcinogen common in groundwater and certain foods (e.g., rice, grains) (EFSA 2009). Evidence from Taiwan, Bangladesh, and Mexico supports an association of high arsenic levels in drinking water (≥50 μg/L) with type 2 diabetes although most studies are cross-sectional (Maull et al. 2012). At low-moderate water arsenic (<50 μg/L), cross-sectional and prospective evidence from the United States, Canada, and Denmark support a possible role of arsenic in diabetes with mixed results (Brauner et al. 2014; Feseke et al. 2015; James et al. 2013; Navas-Acien et al. 2008; Zierold et al. 2004). Most of these studies, however, lack arsenic biomarker data (Brauner et al. 2014; James et al. 2013; Zierold et al. 2004), and some them rely on diabetes registries or diabetes mortality for outcome assessment (D'Ippoliti et al. 2015).

The toxicity of arsenic is influenced by its metabolism (Drobna et al. 2009). After absorption, iAs is metabolized into mono- and di-methylated compounds (MMA and DMA) and the three arsenic forms are excreted in the urine, with DMA being more rapidly excreted via the kidneys (Aposhian and Aposhian 2006; Vahter 2002). Lower methylation capacity, characterized by increased MMA% compared with DMA% in urine, has been identified as a risk factor for several human diseases, including skin lesions, cardiovascular disease, skin cancer, and bladder cancer (Kuo et al. 2017). Increasing evidence also supports the role of arsenic metabolism in type 1 and type 2 diabetes (Grau-Pérez et al. 2016; Mendez et al. 2016; Nizam et al. 2013), including prospective evidence (Kuo et al. 2015). However, contrary to what has been observed for other health outcomes, lower MMA%, and higher DMA% in urine has been related to type 2 diabetes risk in adults (Kuo et al. 2015; Mendez et al. 2016; Nizam et al. 2013). Arsenic methylation is partly determined by genetic

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variants in *AS3MT* (encoding arsenic (III) methyltransferase) (Balakrishnan et al. 2016) and some one-carbon metabolism (OCM) nutrients (Gamble et al. 2006; Spratlen et al. 2017). In particular, randomized clinical trials (Gamble et al. 2006) and observational studies (Kordas et al. 2016; Spratlen et al. 2017) showed that supplementation and intake of folate and other OCM nutrients increased arsenic methylation capacity (decreased iAs% and increased DMA%). In a cross-sectional study in children and adolescents, arsenic metabolism and plasma folate showed an interaction with type 1 (and maybe type 2) diabetes (Grau-Pérez et al. 2016). No studies have evaluated the interaction between arsenic metabolism and OCM on diabetes using a prospective design or in adult populations.

American Indian communities in the United States are affected by disproportionate exposure to arsenic and a high burden of diabetes compared with other U.S. populations. In the Strong Heart Study (SHS), a population-based study of American Indian adults 45–74 y of age, the prevalence of type 2 diabetes at baseline (1989–1991) ranged from 34% in North/South Dakota to 68% in Arizona (Welty and Coulehan 1993), compared with the 21% among U.S. adults 45 and older in 2012 (CDC 2014). In the SHS, arsenic exposure—assessed in urine—was associated with prevalent (Gribble et al. 2012), but not with incident diabetes (Kuo et al. 2015). The lack of a prospective association could be related to a small pool of susceptible participants owing to older age and high burden of diabetes at baseline (Kuo et al. 2015). Arsenic metabolism, in particular lower MMA% and higher DMA%, was associated with both diabetes prevalence and incidence in the SHS (Kuo et al. 2015).

In this study, we evaluated the prospective association of arsenic exposure and metabolism with type 2 diabetes in the Strong Heart Family Study (SHFS), an extension of the SHS that recruited family members of the SHS participants. By including a younger population (median, 36 y of age), the SHFS allows the evaluation of the association between arsenic and diabetes early in the natural history of the disease. We also evaluated the association of arsenic exposure and metabolism with insulin resistance, a key etiopathogenic mechanism underlying type 2 diabetes. Because prediabetes may influence arsenic metabolism and excretion, we conducted an *a priori* sensitivity analysis stratifying by prediabetes condition at baseline. We hypothesized a prospective association between arsenic exposure and metabolism (higher DMA% and lower MMA% in urine) with incident type 2 diabetes and insulin resistance.

Methods

Study Population

The SHFS is a prospective family based cohort study designed to identify genetic and environmental factors for cardiovascular disease and their risk factors in American Indians from 13 communities residing in Arizona, Oklahoma, North Dakota, and South Dakota. Details about design and methodology for SHFS have been published (North et al. 2003). In the SHFS, 3,838 men and women from 96 families have baseline data that were collected in 1998–1999 and 2001–2004, and follow-up data in 2001–2004 (for some participants recruited between 1998–1999) and 2006–2009 (Figure S1). The protocol was approved by the institutional review boards of the Indian Health Service and the participating Indian tribes. All participants gave informed consent.

Participants free of diabetes at baseline and with available urine arsenic measurements were selected for this study ($n = 2{,}453$). Due to tribal request, data from one of the original tribes was not used ($n = 504$). We further excluded participants missing diabetes status at follow-up ($n = 38$), urine creatinine measurements ($n = 1$), baseline values of homeostasis model assessment for insulin resistance (HOMA2-IR) ($n = 25$), and other relevant covariates such as baseline body mass index (BMI), waist circumference, estimated glomerular filtration rate (GFR), smoking status, and *AS3MT* genotype ($n = 47$). As a result, 1,838 participants were included in the present study. Included participants were similar to those who were excluded because of missing data (not shown).

Diabetes and Insulin Resistance Determinations

We determined two study outcomes at the follow-up visit: *a*) incident type 2 diabetes status (yes/no); and *b*) HOMA2-IR (continuous). Incident type 2 diabetes was defined as fasting plasma glucose ≥ 126 mg/dL, self-reported physician diagnosis or self-reported use of insulin or oral diabetes treatment. Similar to other studies (Chow et al. 2013; Juraschek et al. 2013), we estimated the date of diagnosis under the assumption that glucose levels increased at a linear rate between study visits for participants diagnosed based on glucose levels. Impaired fasting glucose (IFG) and normal fasting glucose (NFG) were defined as fasting glucose concentrations between 100 and 126 mg/dL and <100 mg/dL, respectively. Baseline and follow-up HOMA2-IR values were calculated with the computed solved model for HOMA2-IR (Levy et al. 1998) using fasting glucose and insulin values. HOMA2-IR at follow-up was estimated only among people free of incident diabetes because HOMA-IR correlates well with insulin sensitivity in the SHS nondiabetic population (Resnick et al. 2002).

Arsenic Measurements

Spot urine samples collected the morning of the baseline visit were stored at $-70°$C. Total urine arsenic was measured by inductively coupled plasma mass spectrometry (ICPMS) and arsenic species (iAs, MMA, DMA, and arsenobetaine) were measured by high-performance liquid chromatography-ICPMS (HPLC-ICPMS) at the Trace Element Laboratory of Graz University, Austria. The limit of detection (LOD) for all arsenic species was 0.1 μg As/L. Among the 1,838 participants, 197 (10.7%), 57 (3.1%), and 111 (6.0%) participants had urine iAs, MMA, and arsenobetaine concentrations below the LOD, respectively. No participants had DMA concentrations below the LOD. We imputed the concentrations of iAs, MMA, and arsenobetaine in 221 participants with only one of the species undetected using the equation $total\,arsenic = iAs + MMA + DMA + arsenobetaine$. For 64 individuals with two arsenic species undetected, we estimated the arsenic species concentrations as the LOD divided by the square root of 2. Those 64 participants were excluded for arsenic metabolism analyses because it is difficult to estimate arsenic metabolism if arsenic exposure itself is very low and imputation as the LOD divided by the square root of more than one of the species would lead to wrong estimates of the arsenic methylation patterns for those individuals. Therefore, only 1,774 participants were included in arsenic metabolism analyses.

We used the sum of iAs, MMA, and DMA (ΣAs) as a measure of inorganic arsenic exposure and the relative proportions of iAs, MMA, and DMA over the sum of the three (expressed as iAs%, MMA%, and DMA%) as biomarkers of arsenic metabolism.

Other Variables

Information on age, sex, study region (Arizona, Oklahoma, North, Dakota, and South Dakota), educational level, and smoking status was provided in a personal interview. Height, weight, and waist circumference were collected by physical examination using a standardized protocol. BMI was calculated dividing the weight in kilograms by the square of height in meters. Estimated GFR was obtained using the chronic kidney disease

epidemiology equation. Estimates of macro- and micronutrients, including data on folate and other B vitamins (B1, B2, B6, and B12), were measured through a Block 119-item food frequency questionnaire (FFQ). Detailed information about the FFQ has been previously published (Fretts et al. 2012; Spratlen et al. 2017). Information on vitamins B1 and B12, however, was not used for this study because they were not available for most of participants. Urine creatinine levels were measured by an automated alkaline picrate reagent method. We studied effect modification of the associations by rs12768205 in *AS3MT*, the single nucleotide polymorphism (SNP) with the strongest association in a MetaboChip association analysis with iAs%, MMA%, and DMA% in the SHFS (Balakrishnan et al. 2016). SNP genotyping details have been previously published (Balakrishnan et al. 2016).

Statistical Methods

The distribution of ΣAs was divided by urine creatinine to account for urine dilution and log-transformed for the analyses. iAs%, MMA%, and DMA% were analyzed in the original scale. Because iAs%, MMA%, and DMA% sum to 100%, we used a diagram of three axes (triplot) to describe the compositional means of baseline iAs%, MMA%, and DMA% in participants with normal fasting glucose, impaired fasting glucose, and type 2 diabetes at follow-up.

We estimated the hazard ratios of diabetes incidence and the geometric mean ratios (GMR) of HOMA2-IR by urine arsenic exposure levels and arsenic metabolism patterns. Hazard ratios (HR) were evaluated using Cox proportional hazard models with age as time scale and age at baseline treated as staggered entries. For HOMA2-IR analyses, we conducted multilevel models (MLM) in which both HOMA2-IR values at baseline and at follow-up were treated as the outcome and the linear predictor included the interaction of arsenic and time since baseline (in years). Specifically, the time variable included two values for each participant: time = 0 and time = follow-up duration (in years). This analytical strategy allows estimating the GMR of HOMA2-IR by arsenic levels at baseline (time = 0), the corresponding GMR at follow-up (for instance considering 5 y of follow-up, time = 5), and the mean change on the GMR per each year of follow-up (increasing time one unit). In order to account for the lack of independence among family members, we used mixed effects Cox proportional hazard models for HR and linear regression models with generalized estimating equations for GMR. ΣAs was introduced in the models as continuous (comparing participants in the 75th vs. the 25th percentile) and as tertiles (comparing participants in the two highest tertiles vs. the lowest one). The role of arsenic metabolism was evaluated in two ways. First, we entered one arsenic species percentage in the models (conventional approach) and the associations were estimated for a 5% increase in each species in separate models. To address the difficult interpretation of the traditional approach given that a percentage increase of one arsenic species yields to a percent decrease in one or two of the other arsenic species, we entered two arsenic species percentages in the same model (leave-one-out approach), and reported the associations per a 5% increase of one of the included percentages, meaning that the levels of the second arsenic species in the model are fixed and the not-included arsenic species decreases a 5%.

Study region was introduced in all models as a staggered variable. Model 1 was adjusted for sociodemographic variables: sex, age at baseline visit (continuous), and education (<12 y, ≥12 y). Model 2 was further adjusted for traditional cardiovascular risk factors: BMI, waist circumference, smoking status (never, former, current), estimated GFR, and fasting glucose levels at baseline (normal, impaired). Model 3 was further adjusted for relevant determinants of arsenic metabolism that we wanted to evaluate in exploratory analyses: estimated dietary vitamin B2, B6, and folate (continuous) and allelic dosage of rs12768205. All arsenic metabolism models were also adjusted for ΣAs levels. The use of 12 y as a benchmark for educational level categories has been used in other studies conducted in American Indian populations (Dickerson et al. 2012; Moon et al. 2013). To allow for flexible associations, we modeled ΣAs and the arsenic species percentages using restricted cubic splines. We also explored whether the associations of arsenic exposure and metabolism with incident diabetes and HOMA2-IR are modified by participant subgroups by including in the models the interaction term between the arsenic variable and the corresponding subgroup variable.

Several sensitivity analyses were conducted. We reanalyzed models for the association between diabetes incidence and arsenic exposure stratifying by fasting glucose status (normal vs. impaired) at baseline and in models further adjusted for baseline HOMA2-IR levels (shown in main results). Models further adjusted for urine arsenobetaine (log-transformed), intake of certain food groups (meat, rice and cereals intake), or cigarette packs-per-year showed consistent results (not shown). We also checked the robustness of the findings using other ways to account for urine dilution. In particular, analyses with treating urine arsenic in μg/L and adjusting for specific gravity or urine creatinine in statistical models showed almost identical results (not shown). Finally, analyses excluding participants with undetectable iAs or MMA concentrations resulted in nondifferent results (not shown). All analyses were performed with R software (version 3.3.1; R Development Core Team). The statistical significance level was set at $\alpha = 0.05$.

Results

Participant Characteristics

Median [interquartile range (IQR)] age of study participants was 36 (24–47) y and 60% (1,122) were women (see Table S1). Over 10,327 person-years of follow-up, 255 (13.7%) participants developed diabetes (incidence of 24.7 per 1,000 person-years), with no difference by sex. Compared with nondiabetes participants, individuals who developed diabetes were older and more likely to be obese and to have impaired fasting glucose and higher HOMA2-IR at baseline. Diabetes participants also showed a higher dietary intake estimate of folate than participants without diabetes. In particular, overall median levels of estimated intake of vitamin B2, B6, and folate were 1.6, 1.6, and 336 mg/d, respectively. Median (IQR) urine ΣAs, iAs%, MMA%, and DMA% was 4.4 μg/g creatinine (2.9–7.2), 9.5% (6.3–13.8), 14.4% (11.0–18.1), and 75.6% (68.5–81.7), respectively. The median (IQR) of ΣAs before urine creatinine correction was 5.9 (3.6–9.9) μg/L. Participants with incident diabetes had higher baseline urine ΣAs levels and a metabolic profile characterized by lower MMA% and higher DMA% compared with those without diabetes over the follow-up. The compositional means of iAs%, MMA%, and DMA% showed that individuals with incident diabetes and participants with impaired fasting glucose at follow-up had lower MMA% and higher DMA% levels compared with participants with normal fasting glucose at follow-up (Figure 1). Data on the median (IQR) of ΣAs, iAs%, MMA%, and DMA% on participants subgroups are described in Figures S2 and S3.

Arsenic Exposure and Metabolism and Diabetes Incidence

The fully adjusted HR [95% confidence interval [CI)] of incident diabetes comparing participants in the 75th versus the 25th

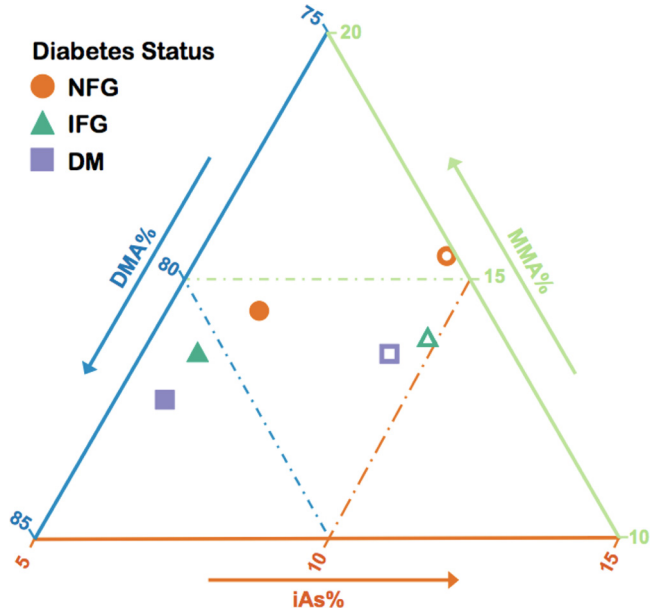

Figure 1. Observed and corrected compositional means of arsenic metabolism biomarkers by type 2 diabetes status at follow-up ($n = 1{,}774$). The triplot shows the compositional means of the arsenic metabolism biomarker distributions in participants with incident diabetes (squares), participants with impaired fasting glucose (triangles), and normal fasting glucose (circles) at follow-up. The unfilled shapes represent the observed means, whereas the solid shapes represent the corrected means after adjustment for sex, age at baseline, baseline education, body mass index, waist circumference, smoking status, estimated glomerular filtration rate, estimated dietary vitamin B2, vitamin B6, and folate and *AS3MT* genotype and baseline fasting glucose. iAs% is presented along the bottom axis, MMA% along the right-hand axis and DMA along the left-hand axis. Compared with participants with NFG at follow-up, individuals with IFG at follow-up, and participants with incident DM had lower MMA% and higher DMA% levels. Note: DM, type 2 diabetes; DMA%, proportion of dimethylarsinate; iAs, inorganic arsenic; IFG, impaired fasting glucose; MMA%, proportion of monomethylarsonate; NFG, normal fasting glucose.

percentile of urine ΣAs was 1.16 (95% CI: 0.94, 1.42) in the complete sample, 1.57 (95% CI: 1.18, 2.08) among participants with normal fasting glucose at baseline, and 0.92 (95% CI: 0.67, 1.21) among participants with impaired fasting glucose at baseline (Table 1, Model 3). Modeling ΣAs as tertiles and restricted cubic splines showed positive and linear associations with incident diabetes that were suggestive but nonsignificant in the complete sample, and significant among normal fasting glucose participants at baseline (Table 1 and Figure 2). No associations were found between arsenic metabolism and diabetes incidence in fully adjusted models (see Table S2 and Figure S4). In interaction analysis, the association between arsenic exposure and incident diabetes was modified by fasting glucose levels at baseline (p-interaction = 0.003) but not by other participant characteristics (see Figure S5). We found no effect modification of the association between arsenic metabolism and incident diabetes by any participant characteristics (see Figure S6).

Arsenic Exposure and Metabolism and HOMA2-IR

We found that baseline ΣAs was positively associated with baseline HOMA2-IR, but negatively associated with HOMA2-IR at follow-up (Table 2 and Figure 3). In particular, in fully adjusted models comparing an IQR increase in ΣAs, the GMR (95% CI) of HOMA2-IR was 1.04 (95% CI: 1.01, 1.08) at baseline and 0.95 (95% CI: 0.92, 0.98) after 5 y of follow-up. For arsenic metabolism, higher MMA% was associated with lower HOMA2-IR both baseline and follow-up. In particular, the fully adjusted GMR (95% CI) of HOMA2-IR after 5 y of follow-up per 5% increase in arsenic metabolism biomarkers when entered individually in the model (conventional approach) was 0.97 (95% CI: 0.95, 0.99) for iAs%, 0.93 (95% CI: 0.91, 0.95) for MMA%, and 1.04 (95% CI: 1.02, 1.05) for DMA% (Table 2). Using the leave-one-out approach, we confirmed that higher MMA% was associated with decreased HOMA2-IR levels both at baseline and follow-up. The GMR (95% CI) of HOMA2-IR after 5 y of follow-up per 5% increase in MMA% was 0.93 (95% CI: 0.90, 0.96) when iAs% decreased a 5%, and 0.93 (95% CI: 0.91, 0.95) when DMA% decreased a 5%. Models with restricted cubic splines showed the dose–response of these associations and confirmed these findings (Figure 3).

The inverse association between MMA% and HOMA2-IR at follow-up was stronger in men (p-interaction = 0.01; Figure 4) and in participants with higher intake of vitamin B2

Table 1. Hazard ratio (95% CI) of incident type 2 diabetes by urinary arsenic concentrations.

Arsenic exposure	Tertile 1	Tertile 2	Tertile 3	75th vs. 25th
ΣAs, μg/g	≤3.3	3.3–5.8	>5.8	7.2 vs. 2.9
Overall sample ($n = 1{,}838$)				
Cases/noncases	65/549	83/530	104/507	252/1,586
Model 1	1 (Reference)	1.17 (0.83, 1.65)	1.36 (0.94, 1.95)	1.19 (0.98, 1.45)
Model 2	1 (Reference)	1.26 (0.89, 1.79)	1.37 (0.94, 1.99)	1.17 (0.95, 1.43)
Model 3	1 (Reference)	1.25 (0.88, 1.76)	1.36 (0.94, 1.98)	1.16 (0.94, 1.42)
Sens.: Model 3 + HOMA2-IR	1 (Reference)	1.19 (0.84, 1.69)	1.26 (0.87, 1.84)	1.12 (0.91, 1.37)
NFG at baseline ($n = 1{,}376$)				
Cases/noncases	30/431	36/422	59/398	125/1,251
Model 1	1 (Reference)	1.13 (0.67, 1.90)	1.86 (1.10, 3.14)	1.55 (1.19, 2.02)
Model 2	1 (Reference)	1.22 (0.72, 2.07)	2.02 (1.17, 3.50)	1.58 (1.19, 2.10)
Model 3	1 (Reference)	1.24 (0.73, 2.10)	2.03 (1.17, 3.50)	1.57 (1.18, 2.08)
Sens.: Model 3 + HOMA2-IR	1 (Reference)	1.14 (0.67, 1.95)	2.04 (1.19, 3.49)	1.63 (1.23, 2.15)
IFG at baseline ($n = 462$)				
Cases/noncases	35/118	47/108	45/109	127/335
Model 1	1 (Reference)	1.42 (0.88, 2.31)	1.08 (0.63, 1.84)	0.98 (0.72, 1.33)
Model 2	1 (Reference)	1.40 (0.85, 2.29)	1.05 (0.60, 1.83)	0.92 (0.67, 1.27)
Model 3	1 (Reference)	1.40 (0.85, 2.29)	1.05 (0.60, 1.83)	0.92 (0.67, 1.27)
Sens.: Model 3 + HOMA2-IR	1 (Reference)	1.35 (0.83, 2.22)	0.96 (0.55, 1.69)	0.87 (0.63, 1.21)

Note: Model 1 stratified by study region and adjusted for sex, age at baseline, and baseline education (<12 y, ≥12 y). Model 2 further adjusted for body mass index (kg/m^2), waist circumference, smoking status (never, former, current smoker), estimated glomerular filtration rate (mL/min per 1.73 m^2) and fasting glucose status at baseline (normal, impaired). Model 2 for NFG and IFG subsets was not adjusted for normal fasting glucose at baseline. Model 3 further adjusted for estimated dietary vitamin B2, vitamin B6, and folate and *AS3MT* genotype. Sensitivity model was adjusted for all Model 3 variables and further adjusted for log-transformed HOMA2-IR values at baseline. CI, confidence interval; HOMA2-IR, homeostasis model assessment for insulin resistance; IFG, impaired fasting glucose; NFG, normal fasting glucose; sens, sensitivity; ΣAs, sum of iAs, MMA, and DMA urine concentrations.

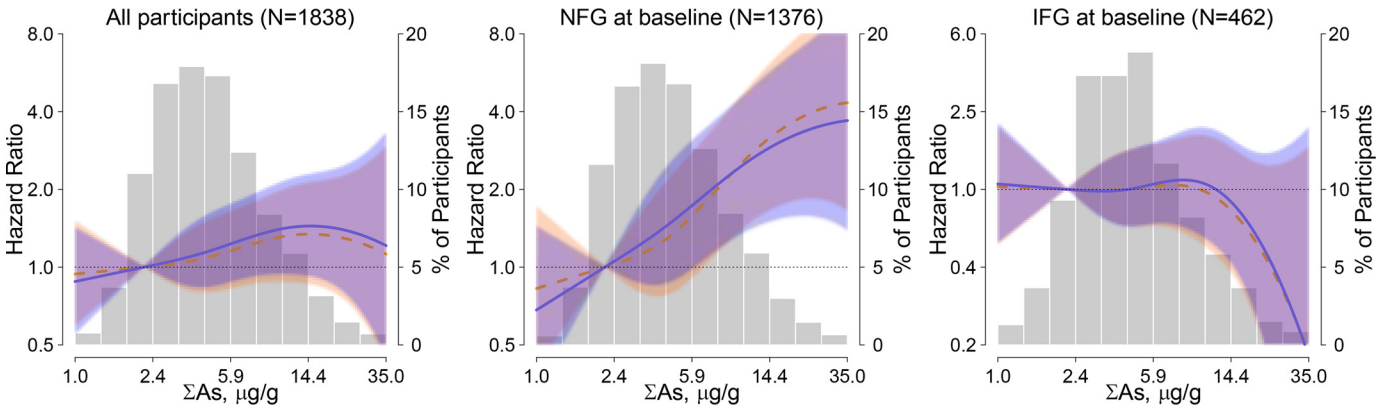

Figure 2. Hazard ratio of incident type 2 diabetes by arsenic exposure in the complete study sample and excluding prediabetes participants at baseline visit. Lines (shaded areas) represent the adjusted hazard ratio (95% confidence interval) of incident type 2 diabetes in the complete sample (left panel), among participants with normal fasting glucose at baseline (middle panel) and among participants with impaired fasting glucose at baseline (right panel), based on restricted cubic splines for log-transformed ΣAs distribution with knots at 10th, 50th, and 90th percentiles. The reference value was set at the 10th percentile. Blue lines (blue shaded areas) represent the estimated hazard ratios in models stratified by study region and adjusted for sex, age at baseline, baseline education (<12 y, ≥12 y), body mass index (kg/m^2), waist circumference, smoking status (never, former, current smoker), estimated glomerular filtration rate (mL/min per 1.73 m^2), estimated dietary vitamin B2, vitamin B6, and folate and *AS3MT* genotype and fasting glucose status at baseline (normal, impaired, only for left panel). Orange dotted lines (orange shaded areas) represent the estimated hazard ratios in models additionally adjusted for log-transformed HOMA2-IR values at baseline. The histograms in the background represent the distribution of ΣAs. The extreme tails of the histograms were truncated because 3 participants had ΣAs levels <1.0 μg/g and 11 had ΣAs levels >35.0 μg/g. Note: HOMA2-IR, homeostasis model assessment for insulin resistance; ΣAs, sum of iAs, MMA, and DMA urine concentrations.

(p-interaction = 0.01), vitamin B6 (p-interaction = 0.002), and folate (p-interaction = 0.03). Moreover, the association between iAs% and DMA% with HOMA2-IR was modified by rs12768205 (p-interaction = 0.03 in both cases).

Discussion

In this study of young adults and adults from American Indian communities in Arizona, Oklahoma, North Dakota, and South Dakota, baseline low-to-moderate arsenic exposure was associated with incident type 2 diabetes among participants with normal fasting glucose at baseline. Arsenic exposure was also associated with increased HOMA2-IR at baseline, but with decreased HOMA2-IR at follow-up. Arsenic metabolism, in particular lower MMA%, either because of higher DMA% or higher iAs%, was associated with higher insulin resistance, suggesting that a metabolic profile characterized by lower MMA% increases vulnerability to develop diabetes. We also found an interaction between OCM nutrients and MMA% and between a genetic variant in *AS3MT*, which encodes the main enzyme involved in arsenic methylation, and iAs% and DMA%, on HOMA2-IR. These findings support that nutritional and genetic factors play a role in increasing susceptibility to arsenic-related diabetes.

Arsenic exposure in humans mainly occurs through drinking water and food (EFSA 2009). More than 140 million people in at least 70 countries are exposed to arsenic above the World Health Organization limit of 10 μg/L in drinking water (Naujokas et al. 2013). Many more millions worldwide are exposed to arsenic in drinking water above 5 μg/L, the water standard in the state of New Jersey, or 1 μg/L, the standard in the Netherlands. Participants of our study are exposed to arsenic concentrations in drinking water below 50 μg/L. Although traditionally the term low-moderate is used for exposure ranging between 10 and 100 μg/L of arsenic in drinking water, we believe that it is becoming less reasonable to use this term for arsenic levels between 50 and 100 μg/L, given the increasing evidence on

Table 2. Geometric mean ratio (95% CI) of HOMA2–IR at baseline and follow-up by inorganic arsenic exposure and arsenic metabolism biomarkers.

Arsenic exposure/biomarker	Biomarker	Baseline effect	Annual change	5-y follow-up
Arsenic exposure (n = 1,838)				
ΣAs, μg/g (6.9 vs. 2.9)		1.04 (1.01, 1.08)	0.98 (0.97, 0.99)	0.95 (0.92, 0.98)
Arsenic metabolism (n = 1,774)				
Conventional approach	iAs%	0.99 (0.97, 1.01)	1.00 (0.99, 1.00)	0.97 (0.95, 0.99)
(5% increase)	MMA%	0.91 (0.88, 0.93)	1.00 (1.00, 1.01)	0.93 (0.91, 0.95)
	DMA%	1.04 (1.02, 1.05)	1.00 (1.00, 1.00)	1.04 (1.02, 1.05)
Leave-one-out approach				
iAs% (5% increase)	MMA% (5% decrease)	1.10 (1.06, 1.14)	1.00 (0.99, 1.00)	1.08 (1.04, 1.12)
	DMA% (5% decrease)	1.01 (0.98, 1.03)	1.00 (0.99, 1.00)	0.99 (0.97, 1.01)
MMA% (5% increase)	iAs% (5% decrease)	0.91 (0.88, 0.94)	1.00 (1.00, 1.01)	0.93 (0.90, 0.96)
	DMA% (5% decrease)	0.91 (0.88, 0.93)	1.00 (1.00, 1.01)	0.93 (0.91, 0.95)
DMA% (5% increase)	iAs% (5% decrease)	1.00 (0.98, 1.02)	1.00 (1.00, 1.00)	1.00 (0.98, 1.02)
	MMA% (5% decrease)	1.09 (1.06, 1.12)	1.00 (1.00, 1.00)	1.09 (1.06, 1.12)

Note: In arsenic exposure analysis, the geometric mean ratio (95% CI) are reported per an increase equal to the IQR in ΣAs distribution. Arsenic metabolism analyses were conducted in two ways. In the conventional approach, each arsenic metabolism biomarker is entered alone in the model and the geometric mean ratios (95% CI) are reported per a 5% increase in that specific biomarker. In the leave-one-out approach, two arsenic metabolism biomarkers are entered together in the model. In that model, a 5% increase in one of the modeled biomarkers corresponds to a 5% decrease of the biomarker that is left outside the model. Models were stratified by study region and adjusted for sex, age at baseline, baseline education (<12 y, ≥12 y), body mass index (kg/m^2), waist circumference, smoking status (never, former, current smoker), estimated glomerular filtration rate (mL/min per 1.73 m^2), fasting glucose status at baseline (normal, impaired), estimated dietary vitamin B2, vitamin B6, and folate and *AS3MT* genotype. All arsenic metabolism models were also adjusted for log-transformed ΣAs concentrations (μg/g). CI, confidence interval; DMA%, proportion of dimethylarsinate; HOMA2-IR, homeostasis model assessment for insulin resistance; iAs%, proportion of inorganic arsenic; IQR, interquartile range; MMA%, proportion of monomethylarsonate; ΣAs, sum of iAs, MMA, and DMA urine concentrations.

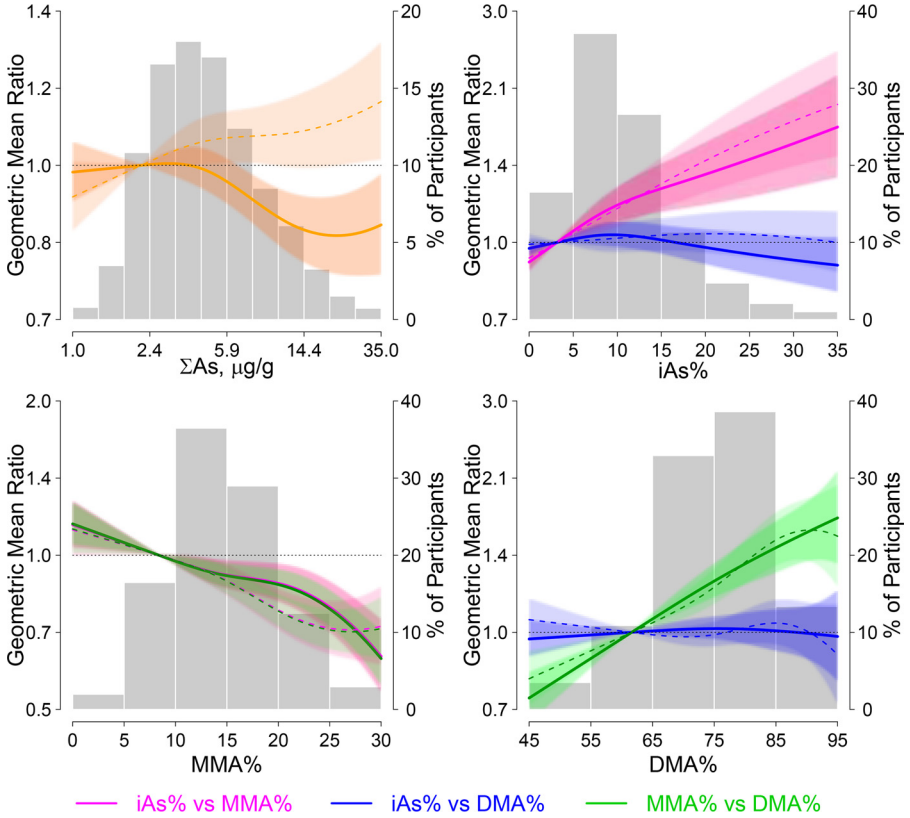

Figure 3. Geometric mean ratio of HOMA2-IR at baseline and follow-up by arsenic exposure and arsenic metabolism biomarkers. Lines represent (shaded areas) adjusted the geometric mean ratio (95% confidence intervals) of HOMA2-IR at baseline (dashed lines) and at follow-up (solid lines) based on restricted cubic splines for log-transformed ΣAs distribution and each arsenic metabolism biomarker with knots at 10th, 50th, and 90th percentiles. The reference value was set at the 10th percentile of each arsenic biomarker distribution. In the top left panel, orange lines (orange shaded areas) represent the geometric mean ratios by ΣAs levels. In the top right panel, pink lines (pink shaded areas) represent the geometric mean ratios by iAs% when it replaces MMA%, and blue lines (blue shaded areas) when it replaces DMA%. In the bottom left panel, pink lines (pink shaded areas) represent the geometric mean ratios by MMA% when it replaces iAs%, and green lines (green shaded areas) when it replaces DMA%. In the bottom right panel, blue lines (blue shaded areas) represent the geometric mean ratios by DMA% when it replaces iAs%, and green lines (green shaded areas) when it replaces MMA%. Models were stratified by study region and adjusted for sex, age, education (<12 y, ≥12 y), body mass index, smoking status (never, former, current smoker), waist circumference, glomerular filtration rate, fasting glucose levels at baseline, estimated dietary vitamin B2, vitamin B6, and folate and *AS3MT* genotype. Arsenic metabolism models were also adjusted for log-transformed ΣAs concentrations. The histograms in the background represent the distributions of each arsenic biomarker (ΣAs, iAs%, MMA%, and DMA%) among the study participants. The extreme tails of the histograms were truncated because 3 participants had ΣAs levels <1.0 μg/g, 11 had ΣAs levels >35.0 μg/g, 9 had iAs% > 35 μg/g, 11 had MMA% > 30 μg/g, 10 had DMA% < 45 μg/g, and 1 had DMA% > 95 μg/g. Note: HOMA2-IR, homeostasis model assessment for insulin resistance; DMA%, proportion of dimethylarsinate; iAs, inorganic arsenic; MMA%, proportion of monomethylarsonate; ΣAs), sum of iAs, MMA, and DMA urine concentrations.

health effects at levels above 50 μg/L and that the new maximum contaminant limit for arsenic changed from 50 to 10 μg/L in 2001. In our study, the median urine ΣAs was 5.9 μg/L (4.4 μg/g creatinine), higher than in the Multi-Ethnic Study of Atherosclerosis (3.1 μg/L) (Jones et al. 2016), a population-based study in six U.S. urban settings, but lower than in the original SHS (10.2 μg/L) (Kuo et al. 2015).

Despite a growing body of evidence on the role of low-moderate arsenic exposure in diabetes, the association has remained unclear because few epidemiologic studies have investigated this association prospectively. In cross-sectional studies, urinary arsenic levels were positively associated with prevalent diabetes in populations from the United States (median urine arsenic ranging 7.1–14.1 μg/L) and Canada (urine arsenic geometric mean 11.4 μg/L) (Feseke et al. 2015; Gribble et al. 2012; Navas-Acien et al. 2008). In Bangladesh, moderate arsenic exposure measured in drinking water (median, 13.9 μg/L among nondiabetic participants) and toenail (median, 2.0 μg/g creatinine among nondiabetic participants) was associated with prevalent diabetes (Pan et al. 2013). In Wisconsin, however, arsenic concentrations in drinking water (median, 2.0 μg/L) were not associated with diabetes prevalence, although diabetes status was self-reported (Zierold et al. 2004). In prospective studies, increased arsenic exposure through drinking water was associated with diabetes risk in rural Colorado (median, 8.0 μg/L) (James et al. 2013) and Denmark (median, 0.7 μg/L) (Brauner et al. 2014), whereas in the original cohort of the SHS the association of urine arsenic (median 10.2 μg/L) with incident diabetes was null (Kuo et al. 2015).

In addition to exposure levels, the toxicity of arsenic depends on its metabolism, which is characterized by a series of methylation steps (Drobna et al. 2009). The mechanisms by which arsenic metabolism may disrupt metabolic function are still uncertain. Recent cross-sectional studies from Mexico and Bangladesh (Mendez et al. 2016; Nizam et al. 2013), and a prospective study from the United States (Kuo et al. 2015), have shown that people with a metabolic profile characterized by lower urine MMA% and higher urine DMA% may have an increased risk of diabetes. In our study we found a significant association between arsenic metabolism and HOMA2-IR but not with incident diabetes. Homeostasis model assessment is a method for assessing insulin resistance using fasting glucose and

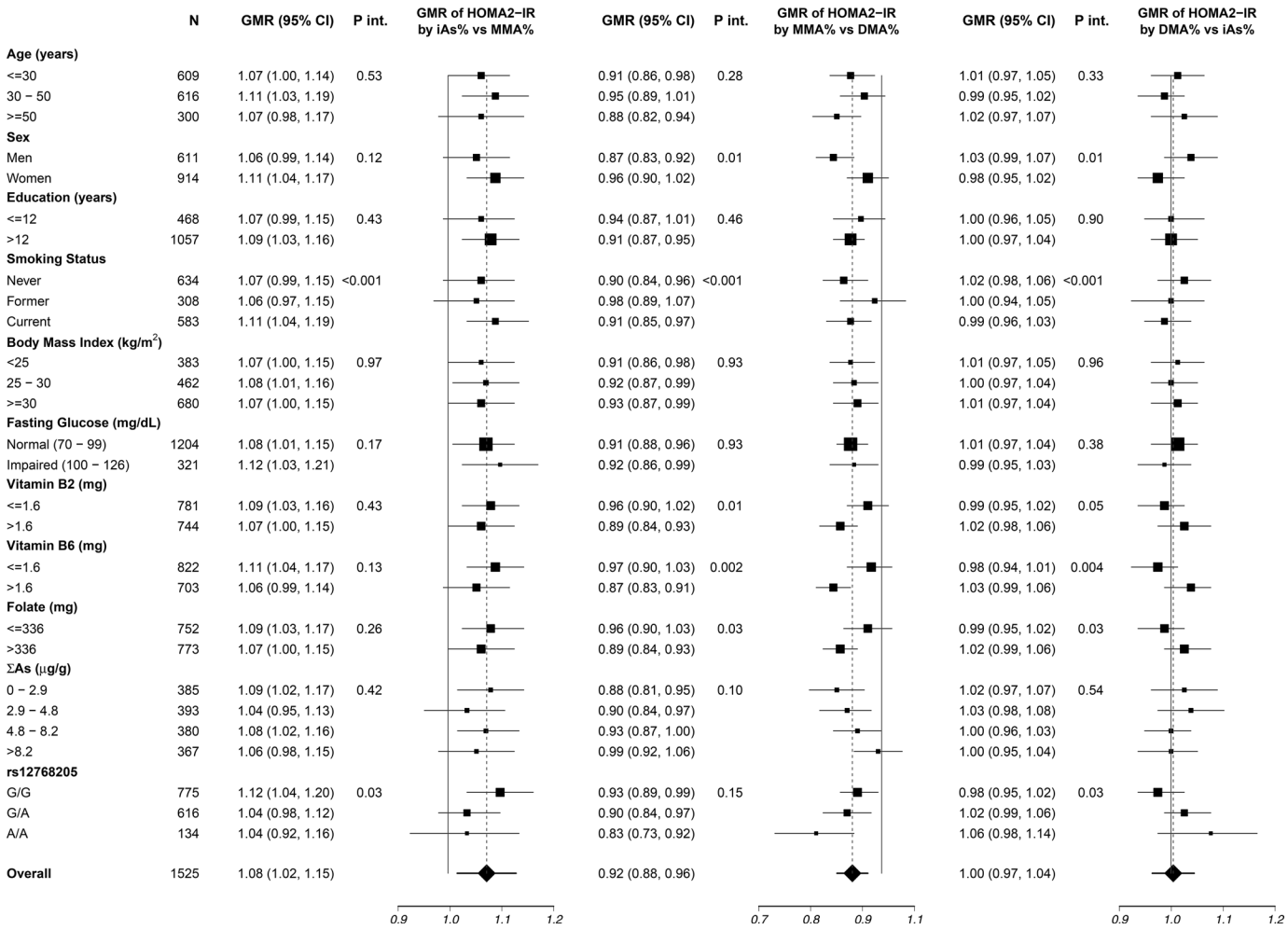

Figure 4. Geometric mean ratio of HOMA2-IR at follow-up by arsenic metabolism biomarkers—interaction analysis ($n = 1{,}525$). The geometric mean ratios of HOMA2-IR were estimated per 5% increase in iAs%, MMA%, and DMA%. Models were stratified by study region and adjusted for sex, age, education, body mass index, smoking status, waist circumference, glomerular filtration rate, ΣAs concentration, estimated dietary vitamin B2, vitamin B6, and folate, and *AS3MT* genotype and fasting glucose levels at baseline. iAs% models were also adjusted for DMA%, then, data in the left panel are the geometric mean ratios when iAs% replaces MMA%. MMA% models were also adjusted for iAs%, then, data in the middle panel are the geometric mean ratios when MMA% replaces DMA%. DMA% models were also adjusted for MMA%, then, data in the right panel are the geometric mean ratios when DMA% replaces iAs%. The area of each marker is proportional to the subgroup sample size. Note: CI, confidence interval; DMA%, proportion of dimethylarsinate; GMR, geometric mean ratio; HOMA2-IR, homeostasis model assessment for insulin resistance; iAs, inorganic arsenic; MMA%, proportion of monomethylarsonate; P int., *p*-interaction; ΣAs, sum of iAs, MMA, and DMA urine concentrations.

insulin measures that is an excellent predictor of diabetes development (Wallace et al. 2004). Few epidemiologic studies have evaluated the association between arsenic exposure and HOMA-IR (Del Razo et al. 2011; Gribble et al. 2012; Lin et al. 2014; Park et al. 2016), and the hypotheses underlying a link between arsenic exposure and insulin resistance are primarily derived from experimental studies (Fu et al. 2010; Palacios et al. 2012). *In vivo* experiments in rats, chronic exposure to arsenic-contaminated water (30 μg/L) significantly increased HOMA-IR values (Palacios et al. 2012). In epidemiologic studies, however, the associations between arsenic exposure and insulin resistance have generally been null or inverse (Del Razo et al. 2011; Gribble et al. 2012; Park et al. 2016), although a study from Taiwan showed a positive relationship (Lin et al. 2014).

No studies evaluating the association between arsenic metabolism patterns and HOMA-IR in human adults have been identified. *As3mt*-knockout mice, which cannot efficiently methylate inorganic arsenic, had higher fasting plasma insulin compared with wild-type mice, regardless of exposure to sodium arsenite (0.1 or 1.0 ppm) (Douillet et al. 2016). Male *As3mt*-knockout mice were also more insulin resistant than female. The association between HOMA2-IR and arsenic metabolism, but not with arsenic exposure, and the interactions between *AS3MT* index SNP and arsenic metabolism biomarkers are consistent with the findings of this *As3mt* knockout model.

The recommended daily allowances (RDAs) of B2, B6, and folate, depending on age and sex, are established as 1.0–1.3 mg/d, 1.2–1.7 mg/d, and 400 mg/d, respectively (NIH 2017), and approximately S-half of the study sample had estimated intake levels of these vitamins above the RDAs (observed median levels were 1.6 for B2 and B6 and 336 mg/d for folate). It is well established in randomized clinical trials (RCT) and observational studies, including evidence from the main cohort of the SHS, that folate and other B vitamins can facilitate the methylation of iAs to DMA, which is more rapidly excreted in urine (Gamble et al. 2006; Kordas et al. 2016; Spratlen et al. 2017). Briefly, those studies found that increased dietary intake of folate and other B vitamins in children (Kordas et al. 2016) and adults (Spratlen et al. 2017) and folic acid supplementation in individuals with low plasma folate (Gamble et al. 2007) were related to lower iAs% and

higher DMA% in urine. In the current study, there were no significant associations between vitamin B intake and arsenic metabolism, although in a small subset with metabolite data available ($n = 59$), *S*-adenosyl methionine (SAM), which is increased by folate levels, was positively related to DMA% and inversely related to iAs% and MMA% (not shown). Individuals with higher estimated B-vitamin intake also showed stronger associations of MMA% and DMA% with HOMA2-IR measured at follow-up. This finding may suggest that participants with high intake of certain B vitamins could be more susceptible to develop diabetes if they also have low MMA% and/or high DMA%. RCTs evaluating folate supplementation on diabetes outcomes have found mixed results, including inverse and increased risks (Gargari et al. 2011; Spoelstra-de et al. 2004), indicating that OCM interventions may not be generalizable to the general population, but may benefit certain subgroups depending on background, nutritional status, and environmental exposures.

The present study has limitations. Due to its observational nature, residual or unmeasured confounding could have occurred. For instance, the true diabetes incidence onset date and biomarker measures of B-vitamin metabolites, which are more reliable than dietary estimates, were not available. In particular, dietary assessment based on FFQ has been associated with an underestimate of intake and it could result in substantial measurement error for OCM nutrients. Although the use of the leave-one-out approach is a strength of the present study, these models could be affected by collinearity owing to the high correlation between arsenic species percentages. In our leave-one-out models, the variance inflation factor coefficients of arsenic species percentages ranged from 1.5 to 3, suggesting a small but not concerning presence of collinearity. Other limitations include the withdrawal to participate in further research from one of the originally participating communities, additional selections bias due to the number of participants excluded because of missing data (although those included and excluded from the study were similar in most participant characteristics), the use of one single urine measurement to assess arsenic exposure and the lack of information about past arsenic exposure, such as *in utero* exposure, which may be relevant for the development of diabetes. This study has several strengths, including the prospective design; the high quality of the protocol and laboratory methods, with the evaluation of arsenic-related phenotypes; the availability of arsenic species concentrations to investigate the role of arsenic metabolism in diabetes; the assessment of arsenic exposure in urine, a biomarker that integrates different exposure sources; and the very low seafood intake in the study population, reducing measurement error related to organic arsenicals in seafood.

Conclusions

In conclusion, in a population exposed to low-moderate arsenic levels through drinking water and food, arsenic exposure was associated with incident diabetes after excluding participants with prediabetes at baseline, but not among those already presenting a prediabetes condition. Arsenic metabolism, in particular low MMA% and high DMA%, was associated with increased HOMA2-IR both at baseline and follow-up. The finding of a possible interaction between arsenic metabolism and OCM nutrients and between arsenic metabolism and genetic variants related to arsenic methylation requires confirmation in larger studies of diabetes related outcomes. The study population is generalizable to other rural and suburban populations in the U.S. characterized by a high burden of diabetes and affected by low-moderate arsenic exposure in drinking water, including American Indian communities. Together with evidence of other health effects related with arsenic, such as cardiovascular and immune diseases, our results provide additional support for enacting and implementing policies that prevent low-moderate arsenic exposure in general populations exposed through water and food in countries around the world, and to inform the ongoing arsenic risk assessment, in particular the evaluation of noncancer end points such as a diabetes diagnosis.

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