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Original Contribution

The Association of Arsenic Exposure and Arsenic Metabolism With the Metabolic Syndrome and Its Individual Components: Prospective Evidence From the Strong Heart Family Study

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Inorganic arsenic exposure is ubiquitous, and both exposure and interindividual differences in its metabolism have been associated with cardiometabolic risk. However, the associations of arsenic exposure and arsenic metabolism with the metabolic syndrome (MetS) and its individual components are relatively unknown. We used Poisson regression with robust variance to evaluate the associations of baseline arsenic exposure (urinary arsenic levels) and metabolism (relative percentage of arsenic species over their sum) with incident MetS and its individual components (elevated waist circumference, elevated triglycerides, reduced high-density lipoprotein cholesterol, hypertension, and elevated fasting plasma glucose) in 1,047 participants from the Strong Heart Family Study, a prospective family-based cohort study in American Indian communities (baseline visits were held in 1998–1999 and 2001–2003, follow-up visits in 2001–2003 and 2006–2009). Over the course of follow-up, 32% of participants developed MetS. An interquartile-range increase in arsenic exposure was associated with a 1.19-fold (95% confidence interval: 1.01, 1.41) greater risk of elevated fasting plasma glucose concentration but not with other individual components of the MetS or MetS overall. Arsenic metabolism, specifically lower percentage of monomethylarsonic acid and higher percentage of dimethylarsinic acid, was associated with higher risk of overall MetS and elevated waist circumference but not with any other MetS component. These findings support the hypothesis that there are contrasting and independent associations of arsenic exposure and arsenic metabolism with metabolic outcomes which may contribute to overall diabetes risk.

American Indians; arsenic; arsenic metabolism; indigenous populations; metabolic syndrome; prospective cohort studies

Abbreviations: ∑As, sum of inorganic and methylated arsenic species; *AS3MT*, arsenite methyltransferase gene; BMI, body mass index; CI, confidence interval; DMA, dimethylarsinic acid; iAs, inorganic arsenic; IQR, interquartile range; MetS, metabolic syndrome; MMA, monomethylarsonic acid; PC, principal component; RR, relative risk.

The metabolic syndrome (MetS) affects one quarter of the global adult population and one third of US adults (1, 2). MetS is characterized by a clustering of abnormalities in waist circumference, triglyceride levels, cholesterol, blood pressure, and glucose levels (3). Persons with MetS have up to a 5-fold greater risk of suffering from coronary heart disease, stroke, or diabetes (2, 4). Efforts are needed to identify and intervene on preventable risk factors for MetS development.

Inorganic arsenic (iAs) is a carcinogen that is associated with increased risk of numerous health effects, including cardiometabolic outcomes (5–22). After ingestion, iAs is converted to mono- and dimethylated arsenic compounds (monomethylarsonic acid

(MMA) and dimethylarsinic acid (DMA)) that are excreted in the urine (23–25). Arsenic metabolism is measured by computing relative percentages of urinary iAs, MMA, and DMA over their sum (iAs%, MMA%, and DMA%). Interindividual differences in arsenic metabolism are related to adverse health outcomes after controlling for arsenic exposure. Due to its relatively shorter half-life and rapid excretion through urine as compared with iAs, higher DMA% is considered a more efficient arsenic metabolism profile and protective against arsenic toxicity. However, increasing evidence indicates that the association between arsenic metabolism and disease is more complex. Indeed, while higher urinary MMA% and lower DMA% have been associated with greater risks of arsenic-related cancer (26–31) and cardiovascular disease (32–34), lower MMA% and higher DMA% have been cross-sectionally associated with higher body mass index (BMI) (35) and prospectively with greater risk of insulin resistance (36), diabetes (16, 37–39), and MetS (11). The mechanism behind these contrasting associations is not clear, yet trends have been consistent across exposure levels and ethnicities (40).

MetS is understudied in the context of arsenic and provides a useful opportunity to understand the conflicting associations with metabolic outcomes reported for arsenic metabolism versus the associations reported with cardiovascular disease, because of the clustering of interrelated but distinct components. To our knowledge, no investigators to date have reported on the relationship of arsenic with both MetS and its individual components. We conducted a comprehensive evaluation of the prospective association of arsenic exposure and metabolism with MetS and its components in the Strong Heart Family Study, a family-based cohort study of members of American Indian tribes from Arizona, Oklahoma, and North/South Dakota. The study communities are affected by a high burden of MetS and are exposed to low-to-moderate levels of arsenic from drinking water and food.

METHODS

Study population

The Strong Heart Family Study investigators recruited 2,919 participants in 1998–1999 and 2001–2003. Participants recruited in 1998–1999 ($n = 428$) had follow-up visits in 2001–2003 and 2006–2009. Participants recruited in 2001–2003 ($n = 2,491$) had a single follow-up visit in 2006–2009. For this study, we included participants who were free of diabetes at baseline and had donated sufficient urine for arsenic analyses ($n = 1,720$). We used urinary arsenic level measured from baseline visits and MetS data from 1–2 follow-up visits. Participants missing information on education, smoking, alcohol intake, BMI, estimated glomerular filtration rate, and urinary creatinine concentration ($n = 18$) were excluded. We further excluded participants missing MetS data ($n = 52$) and prevalent MetS cases ($n = 616$), which resulted in 1,047 participants available for incident MetS analyses. For incidence of individual MetS components, we further excluded participants with prevalent cases of each component under analysis (Figure 1).

All participants provided informed consent. Study protocols were approved by multiple institutional review boards, participating communities, and the Indian Health Service.

Data collection

Baseline and follow-up visits included biospecimen collection, physical examinations, completion of a food frequency questionnaire, and completion of an interview-administered questionnaire (collecting data on age, sex, education, smoking history, alcohol use, and medical history) (41). Measurement of waist circumference, blood pressure, height, and weight, as well as collection of urine and fasting blood samples, was performed during physical examinations by centrally trained nurses following a standardized protocol (42).

Urinary arsenic

Morning spot urine samples were frozen within 1–2 hours of collection and stored at −70°C. For arsenic analyses, up to 1.0 mL of urine from each sample was transported on dry ice to the Trace Element Laboratory at the University of Graz (Graz, Austria). Methods have been described elsewhere (43). Briefly, iAs, MMA, DMA, and arsenobetaine were measured using high-performance liquid chromatography/inductively coupled plasma mass spectrometry. Limits of detection were 0.1 μg/L for all 4 species. Arsenic species concentrations below the limit of detection (<5% for all species) were imputed as the limit of detection divided by $\sqrt{2}$. Arsenobetaine levels were low (median, 0.51 μg/L; interquartile range (IQR), 0.34–1.00), confirming infrequent seafood consumption. Urinary creatinine level was analyzed using automated alkaline picrate methodology.

Metabolic syndrome

Fasting triglycerides, cholesterol, and glucose were measured using enzymatic methods. High-density lipoprotein cholesterol was measured by precipitation with heparin and manganese chloride (42). Systolic and diastolic blood pressure measurements were taken on the right arm after 5 minutes' rest using a Baum mercury sphygmomanometer, and the average of the last 2 of 3 measurements was calculated. Waist circumference was measured at the umbilicus with the participant in a supine position.

MetS was characterized according to the National Cholesterol Education Program Adult Treatment Panel III guidelines (3, 44), as recommended by the Indian Health Service (45), and was defined as 3 or more of the following: elevated waist circumference (≥40 inches (≥102 cm) for men, ≥35 inches (≥89 cm) for women); elevated triglyceride concentration (≥150 mg/dL (or use of medication)); elevated fasting plasma glucose concentration (≥100 mg/dL (or use of medication)); reduced high-density lipoprotein cholesterol concentration (<40 mg/dL for men and <50 mg/dL for women (or use of medication)); and hypertension (systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg (or use of medication)).

Other variables

Estimated daily average dietary intake of 1-carbon metabolism micronutrients, including vitamin B2, vitamin B6, and folate, as well as total caloric intake and percentage of kilocalories derived from fat and protein, were measured at baseline through an interviewer-administered Block 119-item food frequency questionnaire (46).

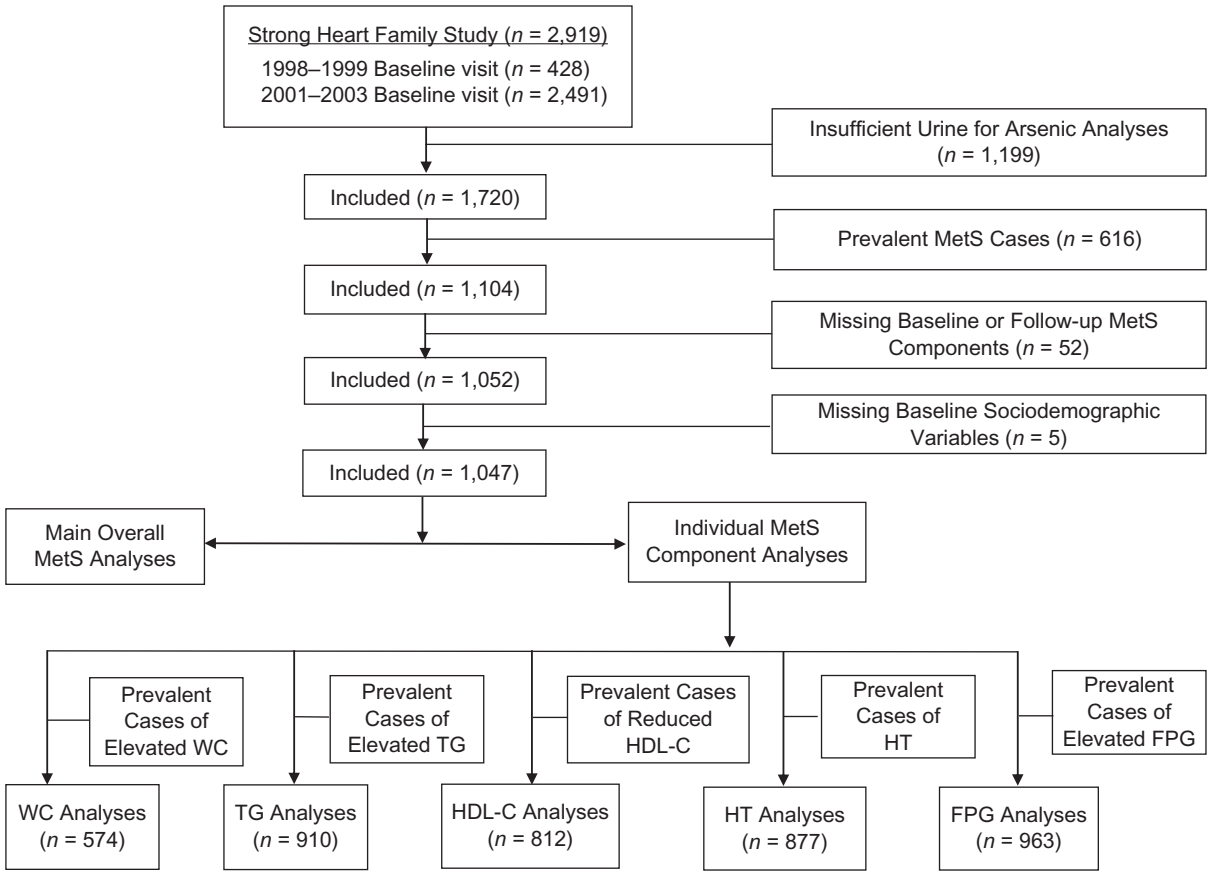

Figure 1. Selection of study participants for an analysis of the associations between arsenic exposure and the metabolic syndrome (MetS) and its components (elevated waist circumference (WC), elevated triglycerides (TG), reduced high-density lipoprotein cholesterol (HDL-C), hypertension (HT), and elevated fasting plasma glucose (FPG)), Strong Heart Family Study, 1998–2003.

Estimated glomerular filtration rate (mL/minute/1.73 m^2; continuous) was calculated from plasma creatinine level, age, and sex using the Chronic Kidney Disease Epidemiology Collaboration formula (47). BMI was calculated as weight in kilometers divided by squared height in meters.

We selected single nucleotide polymorphism rs12768205 on the arsenite methyltransferase gene (*AS3MT*), identified using the Illumina Cardio-Metabo DNA Analysis BeadChip (MetaboChip; Illumina, Inc., San Diego, California), because it showed the strongest association with arsenic metabolism biomarkers in the Strong Heart Family Study (index single nucleotide polymorphism) (48).

Statistical analysis

Data on arsenic exposure, calculated as the sum of inorganic and methylated arsenic species ($\sum$As) in urine, were right-skewed and log-transformed. Arsenic metabolism was computed by dividing each arsenic metabolite concentration over the sum of those species and multiplying by 100 (iAs%, MMA%, and DMA%). Differences in characteristics between participants with and without MetS were determined using Kruskal-Wallis and χ^2 tests for continuous and categorical variables, respectively (Table 1). We assessed the prospective associations of baseline urinary arsenic measures ($\sum$As, iAs%, MMA%, and DMA%) with incident MetS and its individual components. We employed modified Poisson regression with robust variance (49), using generalized estimating equations with an independence working correlation structure to account for family clustering. In the main analyses, we determined the relative risk and 95% confidence interval for overall MetS and each MetS component.

The associations of arsenic exposure with incident MetS and MetS components are reported per IQR increase and with restricted cubic splines to allow for flexibility in the dose-response. For arsenic metabolism, we conducted 3 types of analyses: 1) conventional model, 2) leave-one-out, and 3) principal component (PC). In conventional models, we evaluated each metabolite separately, reporting the relative risk for each outcome per 5–percentage-point increase in each arsenic species. In leave-one-out models, for each arsenic species, we also included one of the species not being evaluated. For example, when evaluating iAs%, if we include MMA%, the regression coefficient estimates the relative risk associated with substituting DMA% for the equivalent iAs% while holding constant MMA% and $\sum$As. This leave-one-out approach has been used previously for arsenic metabolism (37). Finally, we evaluated

Table 1. Baseline Characteristics of American Indian Participants According to Incident Metabolic Syndrome Status at Follow-up, Strong Heart Family Study, 1998–2009

Baseline Characteristic	MetS Status at Follow-up						*P* Value[a]
	Total (n = 1,047)		No MetS (n = 709 (67.7%))		MetS (n = 338 (32.3%))		
	Median (IQR)	%	Median (IQR)	%	Median (IQR)	%	
Age, years	30.7 (20.6–41.8)		28.4 (19.5–40.9)		34.0 (24.4–44.4)		<0.001
Sex							
Female		56.4		56.7		55.6	
Male		43.6		43.3		44.4	0.80
Education, years							
<12		34.7		36.5		30.8	
≥12		65.3		63.5		69.2	0.08
Smoking status							
Never smoker		43.1		43.4		42.3	
Ever smoker		17.7		17.3		18.3	
Current smoker		39.3		39.2		39.3	0.91
Alcohol intake							
Never drinker		11.4		12.4		9.2	
Ever drinker		21.2		20.2		23.4	
Current drinker		67.4		67.4		67.5	0.20
Body mass index[b]	27.4 (23.9–31.6)		26.1 (22.9–30.1)		30.1 (26.5–34.4)		<0.001
Urinary creatinine concentration, mg/dL	1.5 (1.0–2.1)		1.6 (1.0–2.2)		1.5 (1.1–2.1)		0.42
eGFR, mL/minute/1.73 m^2	122 (111–134)		123 (112–134)		120 (108–132)		0.02
∑As, μg/L	6.5 (4.2–10.8)		6.3 (4.2–10.4)		7.0 (4.2–11.7)		0.21
Biomarkers of arsenic metabolism							
iAs%	10.6 (7.5–14.9)		10.9 (7.6–15.3)		9.9 (7.1–13.5)		0.01
MMA%	15.4 (12.2–19.0)		16.0 (12.7–19.8)		14.2 (11.2–17.6)		<0.001
DMA%	73.3 (66.3–78.9)		72.1 (65.6–78.2)		75.1 (69.1–80.5)		<0.001
Components of MetS							
Waist circumference, inches[c]	36.6 (32.7–40.6)		35.4 (31.5–39.0)		39.0 (35.4–43.6)		<0.001
Elevated waist circumference[d]		45.2		38.1		60.1	<0.001
Triglycerides, mg/dL	104 (80.0–135)		96.0 (75.0–125)		119.5 (95.2–146.8)		<0.001
Elevated triglycerides[e]		15.9		12.4		23.1	<0.001
HDL cholesterol concentration, mg/dL[f]	53.0 (44.0–62.0)		54.0 (46.0–63.0)		50.0 (42.0–59.8)		<0.001
Reduced HDL concentration[g]		24.4		20.9		31.7	<0.001
Systolic blood pressure, mm Hg	115 (108–124)		114 (107–122)		119 (111–128)		<0.001
Diastolic blood pressure, mm Hg	74.0 (67.0–80.0)		73.0 (66.0–79.0)		76 (69.0–82.0)		<0.001
Hypertension[h]		18.7		15.8		24.9	0.001
FPG concentration, mg/dL	90.0 (85.0–95.0)		90.0 (85.0–95.0)		92.0 (86.0–96.0)		0.001
Elevated FPG concentration[i]		10.5		10.9		9.8	0.67

Abbreviations: ∑As, sum of inorganic and methylated arsenic species; DMA, dimethylarsinic acid; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HDL, high-density lipoprotein; iAs, inorganic arsenic; IQR, interquartile range; MetS, metabolic syndrome; MMA, monomethylarsonic acid.

[a] *P* values were determined by Kruskal-Wallis test for continuous variables and χ^2 test for categorical variables.

[b] Weight (kg)/height $(m)^2$.

[c] 1 inch = 2.54 cm.

[d] Meets criteria for the elevated waist circumference component of MetS (≥40 inches (≥102 cm) in men and ≥35 inches (≥89 cm) in women).

[e] Meets criteria for the elevated triglyceride component of MetS (≥150 mg/dL (or use of medication)).

[f] To convert HDL cholesterol units to mmol/L, multiply values by 0.0259.

[g] Meets criteria for the reduced HDL cholesterol component of MetS (<40 mg/dL for men and <50 mg/dL for women (or use of medication)).

[h] Meets criteria for the hypertensive component of MetS (systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg (or use of medication)).

[i] Meets criteria for the elevated FPG component of MetS (≥100 mg/dL (or use of medication)).

arsenic metabolism using PC analyses, as recently proposed (48, 50). PC analyses were conducted for arsenic species percentages after normalizing them to have a mean value of 0 and a standard deviation of 1. This method is useful for arsenic metabolism because it removes the interdependence between the 3 biomarkers, with the 2 resulting PCs allowing for potentially more biologically meaningful interpretation, by summarizing primary and secondary methylation steps, respectively (48, 50). Although we cannot be certain that the PCs truly reflect these methylation steps, since arsenic metabolism in the body is still being debated, together with leave-one-out models, this modeling strategy contributes to a more comprehensive picture of how the 3 metabolites interact with respect to their association with health outcomes.

We used progressive adjustments for known determinants of arsenic metabolism and MetS. For ∑As models, model 1 adjusted for urinary creatinine concentration. Model 2 further adjusted for age (years; continuous), sex, region (Arizona, Oklahoma, or North/South Dakota), and education (<12 years or ≥12 years (or above/below the appropriate level of schooling if aged <18 years)). Model 3 further adjusted for BMI (<25, 25–29.9, 30–34.9, 35–39.9, or ≥40), smoking status (never, former, or current smoker), alcohol use (never, former, or current drinker), and estimated glomerular filtration rate. For arsenic metabolism models, model 1 additionally adjusted for log ∑As. In models evaluating waist circumference, BMI was not included as an adjustment factor, for consistency with previous literature (51).

We analyzed possible effect modification of the association between arsenic metabolism and MetS by region, education, smoking status, BMI, ∑As, age, B vitamin intake, and *AS3MT* genotype by adding interaction terms. Interaction *P* values were obtained using Wald tests for multiple coefficients. Participants who were missing information on *AS3MT* genotype ($n = 12$) or vitamin intake ($n = 75$) were excluded from interaction analyses.

We conducted several sensitivity analyses for arsenic metabolism and MetS models. First, we adjusted for number of MetS events present at baseline (0, 1, or 2), since the greater the number of baseline abnormalities the fewer "new" abnormalities there are to be gained for development of MetS. Second, we adjusted for dietary 1-carbon metabolism vitamin intake and estimated daily caloric intake (both log-transformed). Third, we adjusted for percentage of kilocalories derived from fat and protein. Finally, we adjusted for baseline waist circumference in models evaluating arsenic metabolism and elevated waist circumference.

RESULTS

Participant characteristics

Of 1,047 participants without MetS at baseline, 338 (32%) developed MetS over a median follow-up period of 5.3 (IQR, 4.7–6.5) years. The median age was 30.7 years; 56.4% of participants were female and 43.6% were male. Median urinary ∑As concentration was 6.5 μg/L, and median urinary concentrations of iAs%, MMA%, and DMA% were 10.6%, 15.4%, and 73.3%, respectively (Table 1). Compared with participants who remained free of MetS throughout follow-up, those who developed MetS were older, were more educated, had higher BMI and DMA%, and had lower estimated glomerular filtration rate, iAs%, and MMA% at baseline ($P < 0.05$). The first arsenic metabolism PC explained 80.1% of the variance in arsenic species and reflected higher DMA% and lower iAs% and MMA%, possibly representing overall methylation to DMA, or the secondary arsenic methylation step (Table 2). The second arsenic metabolism PC explained 19.9% of the variance and reflected higher MMA% and lower iAs%, independent of DMA%, potentially reflecting the primary methylation step.

Associations of arsenic exposure and metabolism with incident MetS

In adjusted models, ∑As was not associated with incident MetS (per IQR increase in ∑As, relative risk (RR) = 1.03, 95% confidence interval (CI): 0.90, 1.18) (Table 3). For arsenic metabolism, in adjusted conventional models, the relative risks of incident MetS per 5% increase in iAs%, MMA%, and DMA% were 0.94 (95% CI: 0.88, 1.01), 0.87 (95% CI: 0.79, 0.95), and 1.07 (95% CI: 1.02, 1.12), respectively. In iAs-fixed leave-one-out models for MMA% and DMA%, the relative risk remained the same for MMA% (RR = 0.87, 95% CI: 0.79, 0.97) but strengthened to 1.14 (95% CI: 1.03, 1.27) for DMA% (Table 3). Consistently, in flexible dose-response analyses, DMA% showed a linear association with MetS when iAs% was included in the model (see Web Figure 1, available at https://academic.oup.com/aje). The association for DMA%, however, was attenuated in the MMA%-fixed leave-one-out model. In both leave-one-out models for iAs% (fixing either MMA% or DMA%), there remained no significant association with MetS. When modeling arsenic metabolism using PC analysis, an IQR increase in the first arsenic metabolism PC (greater DMA%, lower MMA% and iAs%) was associated with a 1.19-fold (95% CI: 1.06, 1.34) increased risk of MetS, while the second arsenic metabolism PC (greater MMA%, lower iAs%, independent of DMA%) was associated with a 0.93-fold (95% CI: 0.84, 1.03) decreased risk (Table 3).

In interaction analyses of the association of arsenic metabolism with incident MetS according to subgroup characteristics (Table 4), there were interactions between BMI and DMA% (*P* for interaction = 0.03) and between the *AS3MT* rs12768205 polymorphism and MMA% (*P* for interaction = 0.05). The

Table 2. Principal Components of Urinary Arsenic Species Measured in the Strong Heart Family Study, 1998–2009

Variable	Principal Component	
	1	2
Variance in arsenic species explained, %[a]	80.1 (1.6)	19.9 (0.8)
Weight		
iAs%	−0.55	−0.67
MMA%	−0.53	0.74
DMA%	0.64	0.04

Abbreviations: DMA, dimethylarsinic acid; iAs, inorganic arsenic; MMA, monomethylarsonic acid.

[a] Values are presented as mean (standard deviation).

Table 3. Relative Risk of Incident Metabolic Syndrome According to Increases in the Sum of Inorganic and Methylated Arsenic Species and Biomarkers of Arsenic Metabolism (iAs%, MMA%, DMA%, and Principal Components) (n = 1,047), Strong Heart Family Study, 1998–2009

Variable	Model 1[a]		Model 2[b]		Model 3[c]	
	RR	95% CI	RR	95% CI	RR	95% CI
∑As, per IQR increase[d]	1.13	0.99, 1.29	1.05	0.92, 1.19	1.03	0.90, 1.18
iAs% (per 5% increase)						
Conventional model	0.90	0.84, 0.97	0.91	0.85, 0.97	0.94	0.88, 1.01
Leave-one-out models						
MMA% fixed	0.97	0.91, 1.04	0.97	0.90, 1.04	0.98	0.91, 1.05
DMA% fixed	1.21	1.04, 1.40	1.19	1.02, 1.39	1.12	0.96, 1.30
MMA% (per 5% increase)						
Conventional model	0.79	0.72, 0.87	0.80	0.72, 0.88	0.87	0.79, 0.95
Leave-one-out models						
iAs% fixed	0.81	0.73, 0.89	0.81	0.73, 0.91	0.87	0.79, 0.97
DMA% fixed	0.83	0.71, 0.96	0.84	0.72, 0.98	0.89	0.77, 1.04
DMA% (per 5% increase)						
Conventional model	1.11	1.06, 1.17	1.11	1.06, 1.17	1.07	1.02, 1.12
Leave-one-out-models						
iAs% fixed	1.24	1.12, 1.38	1.23	1.10, 1.37	1.14	1.03, 1.27
MMA% fixed	1.03	0.96, 1.10	1.04	0.96, 1.11	1.02	0.95, 1.10
Arsenic metabolism PCs						
PC 1, per IQR increase[e]	1.32	1.17, 1.48	1.32	1.16, 1.49	1.19	1.06, 1.34
PC 2, per IQR increase[f]	0.90	0.82, 0.98	0.91	0.82, 1.00	0.93	0.84, 1.03

Abbreviations: ∑As, sum of inorganic and methylated arsenic species; CI, confidence interval; DMA, dimethylarsinic acid; iAs, inorganic arsenic; IQR, interquartile range; MMA, monomethylarsonic acid; PC, principal component; RR, relative risk.

[a] Model 1 adjusted for log total arsenic (except in models where ∑As was the predictor of interest) and urinary creatinine concentration.

[b] Model 2 further adjusted for sex, region, and education.

[c] Model 3 further adjusted for alcohol intake, smoking status, kidney function, and body mass index.

[d] 10.84 μg/L (75th percentile) vs. 4.20 μg/L (25th percentile).

[e] 1.04 (75th percentile) vs. −0.92 (25th percentile).

[f] 0.50 (75th percentile) vs. −0.38 (25th percentile).

association between DMA% and MetS was weaker with increasing BMI category, and the inverse association between MMA% and MetS was markedly stronger for participants with the rs12768205 AA genotype.

In sensitivity analyses including additional adjustments for baseline number of MetS criteria and B vitamins (Web Table 1), as well as percentage of kilocalories derived from fat and protein (data not shown), we observed consistent results.

Associations of arsenic exposure and metabolism with incident MetS components

In adjusted models, ∑As was associated with elevated fasting plasma glucose concentration (RR = 1.19, 95% CI: 1.01, 1.41) (Table 5) but not with any other MetS component. ∑As remained associated with elevated fasting plasma glucose level in flexible dose-response models (Figure 2).

For arsenic metabolism, the strongest association with individual MetS components was for waist circumference (Figure 3, Table 5). In the conventional model for MMA%, the relative risk of elevated waist circumference for a 5% increase in MMA% was 0.83 (95% CI: 0.76, 0.91), with consistent associations in both leave-one-out models. For DMA%, the relative risk of elevated waist circumference increased from 1.07 (95% CI: 1.01, 1.13) for a 5% increase in DMA% in the conventional model to 1.22 (95% CI: 1.10, 1.34) in the iAs%-fixed leave-one-out model. For iAs%, the only association with waist circumference was in the DMA%-fixed leave-one-out model (RR = 1.25, 95% CI: 1.08, 1.45). In the waist circumference sensitivity analysis that additionally adjusted for baseline waist circumference, results were consistent (data not shown). Clear associations were not observed between arsenic metabolism and other MetS components beyond waist circumference, although in conventional models, generally, higher DMA% and lower MMA% were nonsignificantly associated with higher risk of elevated triglyceride levels, reduced high-density lipoprotein cholesterol level, and hypertension (Figure 3, Table 5).

Table 4. Relative Risk of Incident Metabolic Syndrome According to Increases in Biomarkers of Arsenic Metabolism (iAs%, MMA%, and DMA%) and Levels of Other Risk Factors ($n = 972$), Strong Heart Family Study, 1998–2009[a]

Characteristic	No. of Cases	No. of Controls	Biomarker of Arsenic Metabolism								
			iAs%			MMA%			DMA%		
			RR	95% CI	*P* Value[b]	RR	95% CI	*P* Value	RR	95% CI	*P* Value
Age, years					0.32			0.12			0.18
≤30.0	119	353	0.99	0.90, 1.09		0.93	0.80, 1.09		1.02	0.95, 1.11	
30.1–49.9	140	240	0.88	0.78, 0.99		0.83	0.73, 0.93		1.12	1.04, 1.20	
≥50.0	49	71	0.97	0.78, 1.21		0.73	0.59, 0.89		1.13	0.98, 1.30	
Sex					0.58			0.31			0.40
Male	131	287	0.92	0.83, 1.03		0.8	0.70, 0.92		1.11	1.02, 1.19	
Female	177	377	0.96	0.87, 1.05		0.88	0.78, 1.00		1.06	0.99, 1.13	
Education, years					0.44			0.94			0.59
≤12	97	241	0.98	0.87, 1.09		0.85	0.70, 1.02		1.06	0.97, 1.15	
>12	211	423	0.92	0.84, 1.01		0.84	0.76, 0.94		1.09	1.02, 1.16	
Smoking status					0.97			0.71			0.95
Never smoker	131	284	0.95	0.84, 1.07		0.81	0.70, 0.93		1.09	1.00, 1.19	
Ever smoker	59	119	0.95	0.80, 1.13		0.86	0.69, 1.06		1.07	0.95, 1.20	
Current smoker	118	261	0.93	0.83, 1.04		0.88	0.76, 1.01		1.07	1.00, 1.16	
Body mass index[c]					0.11			0.08			0.03
<25.0 (normal)	50	280	0.82	0.66, 1.02		0.71	0.57, 0.89		1.21	1.06, 1.38	
25.0–29.9 (overweight)	104	207	0.90	0.79, 1.02		0.82	0.70, 0.95		1.11	1.02, 1.20	
≥30.0 (obese)	154	177	1.02	0.92, 1.12		0.92	0.81, 1.05		1.02	0.95, 1.08	
Folate level, μg					0.38			0.38			0.28
<352	143	317	0.98	0.87, 1.09		0.88	0.76, 1.02		1.05	0.97, 1.13	
≥352	165	347	0.91	0.84, 1.00		0.81	0.73, 0.91		1.11	1.04, 1.17	
Vitamin B6 level, mg					0.19			0.71			0.30
<1.7	151	333	0.99	0.89, 1.12		0.86	0.76, 0.98		1.05	0.98, 1.13	
≥1.7	157	331	0.90	0.81, 0.99		0.84	0.73, 0.94		1.11	1.03, 1.17	
Vitamin B2 level, mg					0.51			0.41			0.36
<1.7	154	330	0.96	0.87, 1.07		0.89	0.78, 1.02		1.05	0.98, 1.13	
≥1.7	154	334	0.92	0.84, 1.02		0.83	0.73, 0.93		1.09	1.03, 1.17	
∑As concentration, μg/L					0.44			0.99			0.79
≤4.19	77	168	0.94	0.82, 1.09		0.84	0.69, 1.02		1.08	0.98, 1.20	
4.20–6.57	67	187	1.05	0.90, 1.25		0.86	0.70, 1.06		1.02	0.91, 1.14	
6.58–10.83	82	153	0.90	0.77, 1.05		0.84	0.70, 1.01		1.09	0.99, 1.22	
≥10.84	82	156	0.90	0.78 1.02		0.86	0.74, 1.00		1.09	1.00, 1.19	
AS3MT genotype					0.12			0.05			0.16
GG	154	329	0.98	0.90, 1.06		0.90	0.80, 1.00		1.04	0.98, 1.11	
GA	132	263	0.84	0.73, 0.97		0.80	0.69, 0.94		1.14	1.05, 1.25	
AA	22	72	1.11	1.75, 0.70		0.54	0.35, 0.84		1.15	0.89, 1.49	
Overall	308	664	0.94	0.87, 1.01		0.84	0.76, 0.93		1.08	1.02, 1.14	

Abbreviations: ∑As, sum of inorganic and methylated arsenic species; *AS3MT*, arsenite methyltransferase gene; CI, confidence interval; DMA, dimethylarsinic acid; iAs, inorganic arsenic; MMA, monomethylarsonic acid; RR, relative risk.

[a] Models adjusted for log urinary total arsenic, log urinary creatinine, age, sex, region, smoking status, alcohol intake, B vitamin intake, caloric intake, education, body mass index, and kidney function.

[b] *P* values for interaction were obtained using Wald tests for multiple coefficients.

[c] Weight (kg)/height $(m)^2$.

Table 5. Relative Risk of Incident Components of the Metabolic Syndrome According to Increases in the Sum of Inorganic and Methylated Arsenic Species and Biomarkers of Arsenic Metabolism (iAs%, MMA%, and DMA%), Strong Heart Family Study, 1998–2009

Component, Arsenic Biomarker, and Model	Model 1[a]		Model 2[b]		Model 3[c]	
	RR	95% CI	RR	95% CI	RR	95% CI
Elevated waist circumference ($n = 574$)[d]						
∑As, per IQR increase[e]	0.99	0.86, 1.14	0.99	0.86, 1.14	0.98	0.85, 1.13
iAs%						
Conventional model	0.96	0.88, 1.04	0.97	0.89, 1.06	0.97	0.89, 1.06
LOO model (MMA% fixed)	1.02	0.94, 1.12	1.02	0.94, 1.12	1.03	0.94, 1.12
LOO model (DMA% fixed)	1.29	1.10, 1.51	1.25	1.07, 1.45	1.25	1.08, 1.45
MMA%						
Conventional model	0.80	0.74, 0.88	0.83	0.76, 0.91	0.83	0.76, 0.91
LOO model (iAs% fixed)	0.80	0.72, 0.88	0.82	0.75, 0.91	0.82	0.75, 0.91
LOO model (DMA% fixed)	0.78	0.66, 0.91	0.80	0.69, 0.93	0.80	0.69, 0.93
DMA%						
Conventional model	1.08	1.03, 1.14	1.07	1.01, 1.13	1.07	1.01, 1.13
LOO model (iAs% fixed)	1.26	1.14, 1.39	1.22	1.10, 1.34	1.22	1.10, 1.34
LOO model (MMA% fixed)	0.98	0.89, 1.06	0.98	0.90, 1.06	0.97	0.89, 1.06
Elevated triglyceride concentration ($n = 881$)[f]						
∑As, per IQR increase[e]	0.96	0.81, 1.14	1.00	0.84, 1.19	0.98	0.82, 1.19
iAs%						
Conventional model	0.95	0.86, 1.04	0.93	0.84, 1.03	0.93	0.84, 1.03
LOO model (MMA% fixed)	0.96	0.87, 1.06	0.95	0.86, 1.06	0.94	0.84, 1.05
LOO model (DMA% fixed)	0.99	0.82, 1.18	1.01	0.84, 1.22	0.99	0.81, 1.19
MMA%						
Conventional model	0.95	0.85, 1.06	0.92	0.82, 1.03	0.93	0.83, 1.04
LOO model (iAs% fixed)	0.97	0.86, 1.09	0.94	0.83, 1.06	0.96	0.84, 1.08
LOO model (DMA% fixed)	1.01	0.84, 1.21	0.99	0.82, 1.19	1.01	0.84, 1.23
DMA%						
Conventional model	1.04	0.98, 1.10	1.06	0.99, 1.13	1.05	0.99, 1.13
LOO model (iAs% fixed)	1.03	0.92, 1.16	1.06	0.94, 1.20	1.05	0.92, 1.18
LOO model (MMA% fixed)	1.04	0.94,1.15	1.05	0.94, 1.17	1.06	0.95, 1.18
Reduced HDL cholesterol concentration ($n = 792$)[g]						
∑As, per IQR increase[e]	1.07	0.91, 1.25	0.96	0.81, 1.14	0.95	0.80, 1.14
iAs%						
Conventional model	0.89	0.81, 0.98	0.90	0.82, 0.99	0.92	0.84, 1.02
LOO model (MMA% fixed)	0.94	0.86, 1.04	0.93	0.85, 1.03	0.94	0.85, 1.04
LOO model (DMA% fixed)	1.11	0.93, 1.34	1.04	0.86, 1.25	1.00	0.83, 1.22
MMA%						
Conventional model	0.82	0.73, 0.93	0.87	0.77, 0.98	0.91	0.81, 1.03
LOO model (iAs% fixed)	0.85	0.75, 0.96	0.90	0.79, 1.02	0.94	0.82, 1.07
LOO model (DMA% fixed)	0.90	0.75, 1.08	0.96	0.80, 1.16	1.00	0.82, 1.21
DMA%						
Conventional model	1.11	1.04, 1.18	1.09	1.02, 1.16	1.06	1.00, 1.13
LOO model (iAs% fixed)	1.18	1.04, 1.34	1.12	0.98, 1.27	1.06	0.93, 1.22
LOO model (MMA% fixed)	1.06	0.96, 1.16	1.07	0.97, 1.18	1.06	0.96, 1.17

Table continues

Table 5. Continued

Component, Arsenic Biomarker, and Model	Model 1[a]		Model 2[b]		Model 3[c]	
	RR	95% CI	RR	95% CI	RR	95% CI
Hypertension ($n = 851$)[h]						
∑As, per IQR increase[e]	1.11	0.91, 1.36	1.05	0.90, 1.23	1.03	0.88, 1.20
iAs%						
Conventional model	1.00	0.93, 1.09	0.98	0.90, 1.06	1.00	0.92, 1.08
LOO model (MMA% fixed)	1.00	0.91, 1.09	1.00	0.91, 1.10	1.01	0.91, 1.12
LOO model (DMA% fixed)	0.97	0.80, 1.18	1.09	0.89, 1.33	1.05	0.85, 1.30
MMA%						
Conventional model	1.02	0.90, 1.16	0.92	0.83, 1.03	0.96	0.86, 1.08
LOO model (iAs% fixed)	1.02	0.89, 1.18	0.92	0.81, 1.05	0.96	0.84, 1.10
LOO model (DMA% fixed)	1.03	0.85, 1.25	0.92	0.75, 1.12	0.95	0.77, 1.17
DMA%						
Conventional model	0.99	0.93, 1.05	1.03	0.98, 1.09	1.01	0.96, 1.06
LOO model (iAs% fixed)	0.98	0.85, 1.12	1.08	0.95, 1.23	1.04	0.91, 1.19
LOO model (MMA% fixed)	1.00	0.92, 1.10	1.00	0.91, 1.10	0.99	0.89, 1.09
Elevated fasting plasma glucose concentration ($n = 937$)[i]						
∑As, per IQR increase[e]	1.48	1.28, 1.73	1.25	1.07, 1.46	1.19	1.01, 1.41
iAs%						
Conventional model	1.04	0.95, 1.13	1.01	0.92, 1.10	1.03	0.95, 1.12
LOO model (MMA% fixed)	1.07	0.98, 1.17	1.04	0.95, 1.13	1.03	0.94, 1.12
LOO model (DMA% fixed)	1.21	1.01, 1.44	1.16	0.97, 1.38	1.03	0.86, 1.23
MMA%						
Conventional model	0.92	0.82, 1.03	0.91	0.81, 1.03	1.01	0.90, 1.14
LOO model (iAs% fixed)	0.89	0.78, 1.00	0.90	0.79, 1.02	1.00	0.88, 1.14
LOO model (DMA% fixed)	0.83	0.69, 0.99	0.86	0.72, 1.03	0.97	0.82, 1.16
DMA%						
Conventional model	1.01	0.95, 1.07	1.02	0.96, 1.09	0.98	0.93, 1.04
LOO model (iAs% fixed)	1.13	1.00, 1.27	1.12	0.98, 1.27	1.00	0.88, 1.13
LOO model (MMA% fixed)	0.93	0.86, 1.02	0.97	0.88, 1.05	0.97	0.89, 1.06

Abbreviations: ∑As, sum of inorganic and methylated arsenic species; CI, confidence interval; DMA, dimethylarsinic acid; HDL, high-density lipoprotein; iAs, inorganic arsenic; IQR, interquartile range; LOO, leave-one-out; MMA, monomethylarsonic acid; RR, relative risk.

[a] Model 1 adjusted for log total arsenic (except in models where ∑As was the predictor of interest) and urinary creatinine concentration.

[b] Model 2 further adjusted for age, sex, region, and education.

[c] Model 3 further adjusted for alcohol intake, smoking status, kidney function, and body mass index (except in waist circumference models).

[d] Waist circumference ≥40 inches (≥102 cm) in men and ≥35 inches (≥89 cm) in women.

[e] 10.84 μg/L (75th percentile) vs. 4.20 μg/L (25th percentile).

[f] Triglyceride concentration ≥150 mg/dL (or use of medication).

[g] HDL cholesterol concentration <40 mg/dL for men and <50 mg/dL for women (or use of medication).

[h] Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg (or use of medication).

[i] Fasting plasma glucose concentration ≥100 mg/dL (or use of medication).

DISCUSSION

In American Indian men and women aged ≥14 years from Arizona, Oklahoma, and North/South Dakota, arsenic exposure was associated with increased risk of elevated fasting plasma glucose level but not with MetS or other individual components of MetS. Arsenic metabolism patterns, independent of arsenic exposure, were associated with both incident MetS and elevated waist circumference but not with other components of the syndrome. The relative percentage of MMA appeared to be the main driver behind these associations. The distinct and independent associations of arsenic exposure and arsenic metabolism with MetS and its individual components suggest that these are unrelated phenomena that could be contributing to overall diabetes risk at low levels of arsenic exposure. For arsenic exposure, the association appears to be predominately with hyperglycemia. For arsenic metabolism, the association appears to be mainly with central adiposity. The possible role played by arsenic

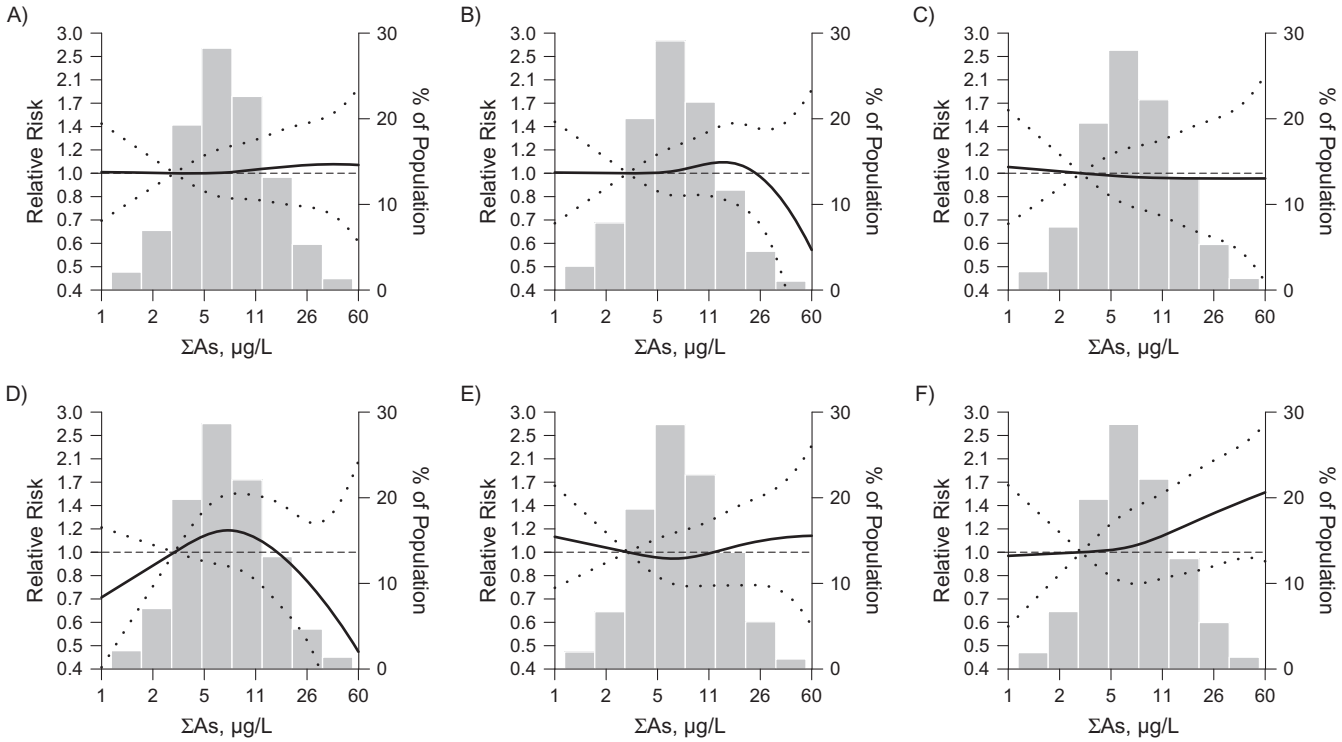

Figure 2. Relative risk of the metabolic syndrome and its individual components according to the sum of inorganic and methylated arsenic species (∑As), Strong Heart Family Study, 1998–2009. ∑As was calculated as the sum of urinary inorganic arsenic, monomethylarsonic acid, and dimethylarsinic acid concentrations. The solid black lines represent adjusted relative risks of: incident metabolic syndrome, defined as meeting 3 or more of the individual criteria (A); elevated waist circumference, defined as ≥40 inches (≥102 cm) in men and ≥35 inches (≥89 cm) in women (B); elevated triglyceride level, defined as ≥150 mg/dL (or use of medication) (C); reduced high-density lipoprotein cholesterol level, defined as <40 mg/dL for men and <50 mg/dL for women (or use of medication) (D); hypertension, defined as systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg (or use of medication) (E); and elevated fasting plasma glucose level, defined as ≥100 mg/dL (or use of medication) (F). Relative risks were calculated using restricted cubic splines for ∑As with knots at the 10th, 50th, and 90th percentiles of the ∑As distribution and were adjusted for log urinary creatinine, age, sex, body mass index (excluding waist circumference models), educational level, region (Arizona, Oklahoma, or North/South Dakota), smoking status, alcohol intake, and kidney function. The referent was set at the 10th percentile of the ∑As distribution. Dotted lines show 95% confidence intervals. Histograms represent the distribution of log-transformed ∑As values.

exposure in hyperglycemia and by arsenic metabolism in central adiposity could be underlying mechanisms for the observed associations between arsenic and diabetes in multiple populations.

MetS has been identified as the driving force behind the global epidemics of diabetes and cardiovascular disease (2). The incidence of metabolic conditions continues to rise, with projections for global diabetes incidence alone to double by 2025 (2). Similar trends are evident in the United States (1, 52). While the National Health and Nutrition Examination Survey does not monitor trends in American Indians, Russell et al. (45) reported a MetS prevalence of up to 63% in an older American Indian cohort (ages 45–74 years). American Indians/Alaska Natives are reported to have the highest age-adjusted prevalence of diabetes in the United States (53). To our knowledge, our study was the first to evaluate the incidence of MetS from adolescence onwards among American Indians. With a median age of 30 years, our baseline MetS prevalence of 36% and incidence of 32% over a median of 5.3 years of follow-up is undoubtedly high. The elevated risks of diabetes and MetS among American Indians as compared with other US populations may be influenced in part by genetic background; however, environmental factors, including disproportionate exposure to arsenic in drinking water, have also been implicated (54–58). Our study supports the hypothesis that exposure to environmental contaminants, particularly arsenic, could play a role.

The association between higher ∑As and elevated fasting plasma glucose level supports previous experimental and epidemiologic evidence suggesting that iAs may have a diabetogenic effect. Although the mechanisms are not fully understood, experimental studies have shown that arsenic may induce diabetes by inhibiting insulin signaling and insulin-dependent glucose uptake, with both microRNAs and mitochondria being proposed as potential mechanistic links (21, 22, 59, 60). Epidemiologic studies have found associations between arsenic and diabetes at both high (≥100 μg/L) (22, 61–64) and moderate (<100 μg/L) (16–18, 38, 65–68) exposure levels, although findings for low levels are mixed (37, 69–71).

The association between arsenic exposure and MetS, as well as other components of MetS beyond fasting plasma glucose, has been less studied. In contrast to our null findings on the relationship between ∑As and MetS, 2 studies conducted in highly exposed Taiwanese populations found a positive association (11, 12). Similarly, studies conducted in high-exposure

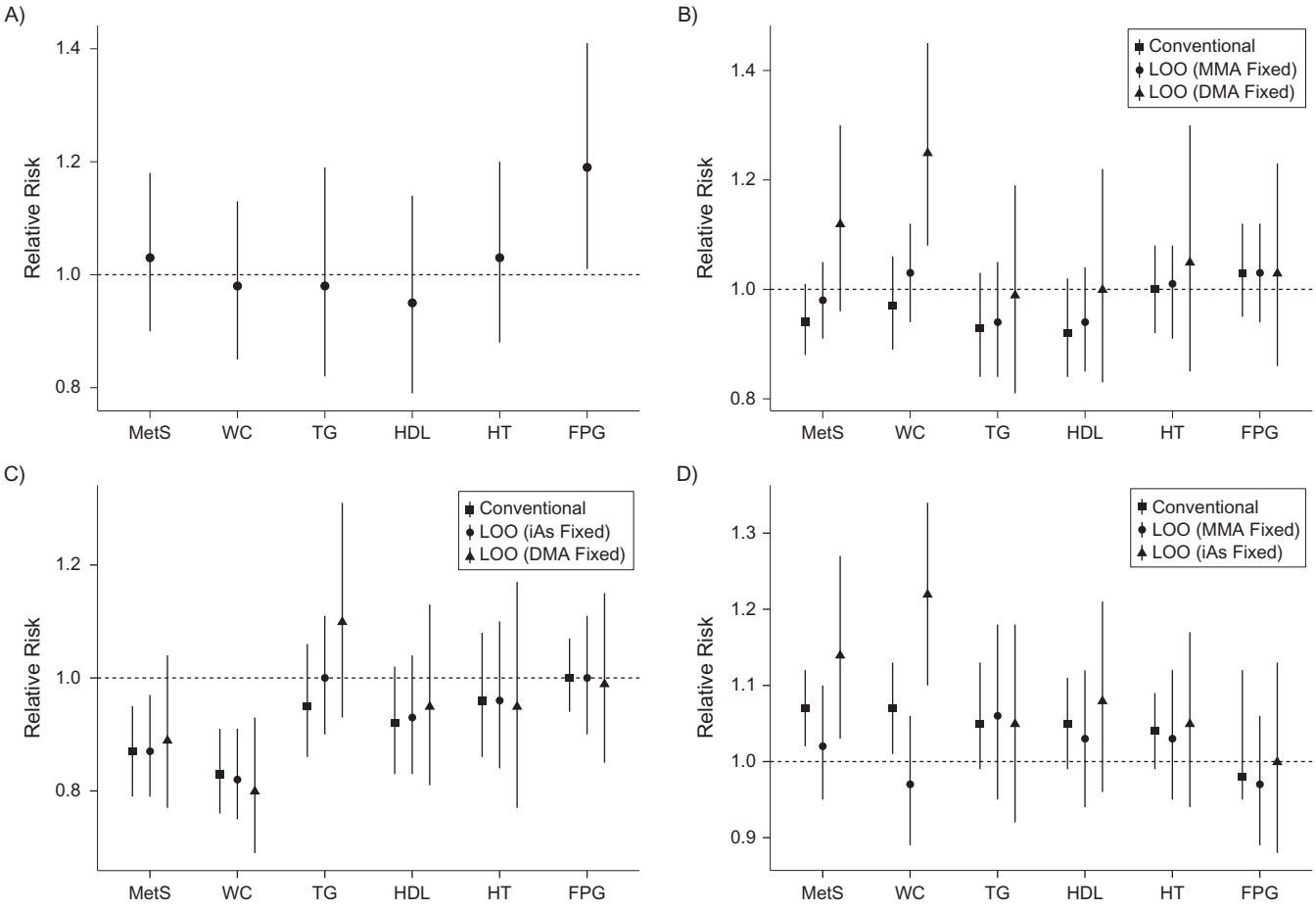

Figure 3. Relative risk of the metabolic syndrome (MetS) and its individual components according to the sum of inorganic and methylated arsenic species (∑As) and per 5% increase in biomarkers of arsenic metabolism, Strong Heart Family Study, 1998–2009. ∑As was calculated as the sum of urinary inorganic arsenic (iAs), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) concentrations. Part A shows the relative risk of incident MetS and its individual components (elevated waist circumference (WC; ≥40 inches (≥102 cm) in men and ≥35 inches (≥89 cm) in women), elevated triglyceride (TG) level (≥150 mg/dL (or use of medication)), reduced high-density lipoprotein (HDL) cholesterol level (<40 mg/dL for men and <50 mg/dL for women (or use of medication)), hypertension (HT; systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg (or use of medication)), and elevated fasting plasma glucose (FPG) level (≥100 mg/dL (or use of medication)) per interquartile-range increase in ∑As. Points (●) represent relative risk, and vertical lines represent 95% confidence intervals. Parts B–D show the relative risk of MetS and its individual components per 5% increase in percentage of iAs (B), percentage of MMA (C), and percentage of DMA (D). The 3 different types of points in panels B–D reflect the 3 different models used to estimate relative risk: the conventional model (■) and 2 leave-one-out (LOO) models (● and ▲). Relative risks were adjusted for log ∑As (excluding exposure models), log urinary creatinine, age, sex, body mass index (excluding waist circumference models), educational level, region (Arizona, Oklahoma, or North/South Dakota), smoking status, alcohol intake, and kidney function.

regions suggest that arsenic exposure is associated with increased risk of hypertension (14, 20, 32, 72, 73), with mostly null findings in low-exposure populations (16, 19, 74), which is consistent with our results for hypertension. A few studies have suggested an association between arsenic exposure and triglycerides (16, 75) and high-density lipoprotein cholesterol (76, 77), in contrast to our null findings. However, epidemiologic studies evaluating low-to-moderate arsenic and triglyceride levels, high-density lipoprotein cholesterol, or hypertension have been cross-sectional. Finally, the association between arsenic exposure and waist circumference has not been evaluated before. In our study, we found no association.

For arsenic metabolism, our finding that lower MMA% and higher DMA% are prospectively associated with both increased risk of incident MetS and elevated waist circumference is consistent with studies that have evaluated these relationships (11, 35) and related outcomes such as diabetes (16, 37, 39, 68) and BMI (35, 78, 79), although most have been cross-sectional. To our knowledge, our evaluation of arsenic metabolism and MetS in a population exposed to low-to-moderate levels of arsenic is novel, as is our prospective assessment of the association between arsenic metabolism and waist circumference. Further, the results from our leave-one-out models add to prospective evidence showing that greater DMA% due to reductions in MMA% is related to incident diabetes (37).

Mechanistic understanding of the increased risk reported for metabolic outcomes with lower MMA% is limited. MMA and DMA in this study, as in most epidemiologic studies, include

pentavalent and trivalent forms. Trivalent forms oxidize rapidly to pentavalent forms in urine, making them indistinguishable (80). Trivalent methylated arsenicals are considered the most toxic metabolites, and MMA^{III} has been proposed as a mechanism for the associations between higher MMA% and increased risk of cancer, cardiovascular disease, and skin lesions (21, 26, 34). In turn, some studies have suggested that the association between higher DMA% and increased risk of metabolic outcomes may be due to DMA^{III} (16, 37, 68). Other studies have suggested that confounding by diet may explain these associations. For example, a nutritionally sufficient diet with high fat and protein intake may enhance arsenic metabolism efficiency while also having adverse metabolic effects (39). In our study, however, adjusting for percentage of kilocalories derived from protein and fat did not affect results. Diet has also been suggested to play a role through 1-carbon metabolism (37). One-carbon metabolism, regulated by B vitamins, is responsible for providing methyl groups necessary for methylation reactions in arsenic metabolism and could be driving the association between arsenic metabolism and metabolic outcomes (25). However, we saw no evidence of confounding or effect modification by estimated intake of B vitamins (folate, vitamin B2, and vitamin B6).

In pregnant women, DMA% increases as women progress through pregnancy (81, 82). Because adiposity increases during pregnancy, it has been suggested that adiposity may be driving shifts in arsenic metabolism patterns (35). Our finding that waist circumference had the strongest associations with arsenic metabolism supports the possibility of adiposity's playing a key role in the pathway between arsenic metabolism and metabolic outcomes. Our association was prospective, and it remained after adjustment for baseline waist circumference, suggesting that adiposity may be a consequence of arsenic metabolism, not a cause; still, we cannot discard the possibility that reverse causality explains the association. Notably, however, we saw significant interactions of DMA% and MetS with BMI, with an increasing strength of association with lower BMI. This could be interpreted as evidence against reverse causality, as we are seeing an association between higher DMA% and MetS among persons with normal BMI at baseline. In a previous study in this cohort, participants with the *AS3MT* rs12768205 AA genotype had lower MMA% (48). We observed an interaction between MMA% and *AS3MT* genotype, with risk of MetS being further reduced with increasing MMA% with each additional A allele, suggesting that the inverse association between MMA% and MetS is stronger among persons with genetically lower MMA%.

Our study strengths included a broad age distribution, a well-established cohort, high-quality laboratory methods, and consideration of genetic contributions. As in most epidemiologic studies, we were unable to differentiate between trivalent and pentavalent forms of MMA and DMA. We confirmed that seafood intake in our population was infrequent and adjusted for intake of B vitamins; however, we cannot discount the potential for remaining confounding by food sources. Further, we did not have information on vitamin B12, an important vitamin in the 1-carbon metabolism pathway. Because we did not have repeated measurements of arsenic exposure and metabolism, we could not evaluate how changes in adiposity and fasting glucose were related to changes in arsenic exposure over time; therefore, we cannot totally exclude the possibility of reverse causality, despite the study's prospective design.

In conclusion, our findings support previous evidence that arsenic has diabetogenic effects even at low-level exposures and may be contributing, in part, to the high burden of diabetes in arsenic-exposed populations. Further, our results suggest that disruption of glucose regulation maybe a key pathway driving the association between low-level arsenic exposure and cardiometabolic outcomes. In addition, we found that arsenic metabolism patterns, specifically lower MMA%, are prospectively associated with increased risk for the development of MetS and elevated waist circumference. These findings support the importance of preventing low-level arsenic exposure and the need to better elucidate mechanisms underlying the contrasting and independent associations of arsenic exposure and metabolism with MetS.

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