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The Association of Arsenic Exposure and Arsenic Metabolism with All-cause, Cardiovascular and Cancer Mortality in the Strong Heart Study

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Abstract

The effect of low-moderate levels of arsenic exposure and of arsenic metabolism on mortality remains uncertain. We used data from a prospective cohort study in 3,600 men and women aged 45 to 75 years living in Arizona, Oklahoma, and North and South Dakota. The biomarker of inorganic arsenic exposure was the sum of urine inorganic (iAs), monomethylated (MMA) and dimethylated (DMA) arsenic compounds (ΣAs) at baseline. The proportions of urine iAs, MMA

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and DMA over the ΣiAs, expressed as iAs%, MMA%, and DMA%, respectively, were used as biomarkers of arsenic metabolism. Arsenic exposure and arsenic metabolism were associated with all-cause, cardiovascular, and cancer mortality. For each interquartile range (IQR) increase in ΣAs (12.5 μg/L, overall range 0.7-194.1 μg/L), the adjusted hazard ratios (aHRs) were 1.28 (95% CI 1.16-1.41) for all-cause mortality, 1.28 (1.08-1.52) for cardiovascular mortality and 1.15 (0.92-1.44) for cancer mortality. The aHR for mortality for each IQR increase in MMA%, when iAs% is decreasing, was 1.52 (95% CI 1.16-1.99) for cardiovascular disease, 0.73 (0.55-0.98) for cancer, and 1.03 (0.90-1.19) for all-cause mortality. These findings at low-moderate levels of arsenic exposure highlight the need to implement public health measures to protect populations from involuntary arsenic exposure and for research to advance the biological and clinical understanding of arsenic-related health effects in general populations.

Keywords

American Indians; arsenic; arsenic species; arsenic methylation; arsenic metabolism; mortality; cardiovascular disease; cancer; Strong Heart Study

Background

Inorganic arsenic (iAs) exposure is a major public health problem worldwide (World Health Organization (WHO) 2010). Indeed, chronic exposure to a wide range of iAs levels, through both water and food, has been associated with a variety of chronic diseases including various forms of cancer (Chiou et al. 1995), cardiovascular disease (CVD) (Moon et al. 2012; Navas-Acien et al. 2005), diabetes (Maull et al. 2012; Navas-Acien et al. 2008), and kidney dysfunction (Zheng et al. 2013). Chronic arsenic exposure has also been related to increased mortality, including all-cause (Argos et al. 2010), cancer (Hopenhayn-Rich et al. 1998; Yang et al. 2004), and CVD (Chen et al. 2011; Lewis et al. 1999; Moon et al. 2013; Yang et al. 2004) mortality in many parts of the world including Argentina, Bangladesh, Chile, Taiwan, and the USA. Most studies used arsenic concentrations from well water or individual urine total arsenic concentrations as primary exposure matrices. Few studies, if any, have systematically evaluated the role of arsenic metabolism in all-cause and disease-specific mortality.

In humans, the average distribution of arsenic metabolites in urine is 10-30% iAs, 10-20% monomethylarsonate [MMA], and 60-80% dimethylarsinate [DMA], with substantial inter-individual variation (Hopenhayn-Rich et al. 1996; Loffredo et al. 2003). Higher MMA% and lower DMA% in urine have been related to increased risk of various cancers (Chung et al. 2013; Kuo et al. 2017; Steinmaus et al. 2010) and CVD (Huang et al. 2009; Kuo et al. 2017; Wu et al. 2006), although some studies showed inconsistent associations. In addition, recent studies have connected increased urine DMA% with increased prevalence of diabetes (Kuo et al. 2017; Nizam et al. 2013) and adiposity (Gribble et al. 2013). Possible mechanisms underlying a differential role for arsenic methylation patterns on disease outcomes could be related to one-carbon metabolism and methylation dysregulation (Hall and Gamble 2012; Niedzwiecki et al. 2013). Understanding how arsenic methylation capacity is associated with mortality risks and whether the association is different by cause of death could help increase

our understanding of the mechanisms of arsenic toxicity and subsequently help with arsenic risk assessment.

In this study, we expand the work of previous publications from the association of arsenic exposure with cancer mortality (Garcia-Esquinas et al. 2013) and or arsenic with cardiovascular incidence (Moon et al. 2013) by evaluating the association of both arsenic exposure and arsenic metabolism with the risk of mortality, including all-cause, CVD and cancer mortality, in the Strong Heart Study (SHS), a large population-based prospective cohort with up to 20 years of follow-up conducted in American Indian communities exposed to low-moderate arsenic exposure levels through drinking water and food (Lee et al. 1990; Moon et al. 2013). We also evaluated whether the association between arsenic metabolism and mortality was independent of arsenic exposure levels.

Methods

Study population

The Strong Heart Study is a population-based cohort study that examined risk factors of CVD mortality and morbidity in American Indians from Arizona, Oklahoma and North and South Dakota. Overall, 4,549 men and women aged 45-74 years of age were enrolled between 1989 and 1991 (Lee et al. 1990). All eligible individuals were invited to participate in Arizona and Oklahoma, however a cluster sampling procedure was applied in North and South Dakota (Lee et al. 1990; Stoddart et al. 2000). The overall participation rate was 62% (Stoddart et al. 2000). The study population was stable during the follow-up period due to low migration rates and strong cultural and community links among SHS participants (Howard et al. 1996). Compared with nonparticipants, participants were similar in age, body mass index (BMI), and prevalence of self-reported diabetes but were more likely to be female and to have self-reported hypertension (Stoddart et al. 2000). The Indian Health Service, institutional review boards, and participating tribes approved the study protocol. All participants provided informed consent.

For this study, we used data from 3,973 participants with sufficient urine samples at the baseline visit for arsenic measurements. We then excluded 228 participants with one or more arsenic species data below the limit of detection (0.10 μg/L) as arsenic metabolism cannot be evaluated at undetectable arsenic exposure levels. We also excluded 145 participants who were either missing: smoking status (n=5), education level (n=2), alcohol drinking status (n=8), body mass index (n=16), waist-to-hip (WHR) ratio (n=26), hypertension (n=15), estimated glomerular filtration rate (eGFR) (n=66), and fasting glucose level (n=7) at baseline. Using our inclusion criteria, our study consisted of 3,600 participants. Participants who did not meet our inclusion criteria were similar to those participants who did not meet the criteria (data not shown).

Data collection

The baseline visit included a personal interview, physical examination, fasting blood test, and spot urine sample collection (Lee et al. 1990). Sociodemographic (age, sex, and education) and lifestyle (smoking and alcohol status) information was collected by trained

and certified interviewers using standardized questionnaires (Lee et al. 1990). Detailed procedures of clinical and laboratory examinations have been previously published (Lee et al. 1990). Briefly, physical examination was conducted by centrally trained nurses and medical assistants following a standardized protocol and included height, weight, waist and hip circumferences, and systolic and diastolic blood pressure (Lee et al. 1990).

Spot urine samples were collected in the morning and were frozen within 1 to 2 hours of collection. The biospecimens were stored at −70°C or lower before analyses (Lee et al. 1990). Urine creatinine and specific gravity levels were measured by an automated alkaline picrate method and Leica TS 400 total solid refractometer (Leica Microsystems, Buffalo, USA), respectively (Lee et al. 1990). Serum creatinine was measured using an automated alkaline-picrate rate method (Roche Diagnostics, Basel, Switzerland) using Hitachi 717 platform (Hitachi Ltd., Tokyo, Japan) (Zheng et al. 2015). eGFR at baseline was derived from the 4-variable isotope dilution mass spectrometry Modification of Diet in Renal Disease Study equation (Levey et al. 1999).

Urine arsenic measurements

Arsenic species concentrations were determined from urine samples collected at baseline by high-performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC-ICP-MS) (Agilent 1100 HPLC and Agilent 7700x ICP-MS, Agilent Technologies, Santa Clara, California). Arsenic speciation can differentiate species directly related to iAs exposure (arsenite, arsenate, MMA, and DMA) from those related to organic arsenicals such as arsenobetaine, found in seafood, and which are generally considered nontoxic. (National Research Conuncil 1999). The limit of detection (LOD) for iAs, MMA, DMA, and arsenobetaine plus other arsenic cations was 0.1 μg/L. The percentages of participants with concentrations below the LOD were 0.03% for total arsenic, 5.2% for iAs, 0.8% for MMA, 0.03% for DMA, and 2.1% for arsenobetaine and other arsenic cations. An in-house reference urine and the Japanese National Institute for Environmental Studies No. 18 Human Urine were analyzed together with the samples, as controls. Interassay coefficients of variation for total arsenic, iAs, MMA, DMA and arsenobetaine and other arsenic cations for the in-house reference urine were 4.4%, 6.0%, 6.5%, 5.9%, and 6.5%, respectively. Detailed analytic methods and associated quality control procedures for arsenic analysis have been described elsewhere (Scheer et al. 2012).

Arsenic exposure and arsenic metabolism

We used the sum of urine iAs (arsenite and arsenate) and methylated arsenic species (MMA and DMA) as the biomarker of iAs exposure from multiple sources (Hughes 2006; Marchiset-Ferlay et al. 2012; National Research Conuncil (NRC) 1999). Urine arsenic concentrations were divided by urine creatinine concentrations to account for urine dilution and expressed as μg/g creatinine. Urine concentrations of arsenobetaine and other arsenic cations were very low with a median 0.68 μg/g creatinine (interquartile range [IQR] 0.41 to 1.54), confirming that seafood intake was low in this sample, and indicating that DMA mainly came from iAs exposure (Navas-Acien et al. 2011).

To assess arsenic metabolism, we used the proportions of urine iAs (arsenite and arsenate), MMA and DMA over the sum of inorganic and methylated species, expressed as iAs%, MMA%, and DMA% respectively, to evaluate arsenic metabolism. Urine arsenic concentrations and arsenic metabolism biomarkers (relative distribution of arsenic species in urine) in the Strong Heart Study population were stable over a 10-year period between 1989-1991 and 1998-1999, reflecting the appropriateness of a single urine arsenic sample to represent long-term arsenic exposure and metabolism in this population (Navas-Acien et al. 2009).

Mortality follow-up

Study participants were followed from the date of the baseline examination until the date of death or December 31st, 2008, whichever occurred first. The follow-up period was selected since arsenic levels in drinking water were stable through those years, but changed after the implementation of the arsenic maximum contaminant level in 2009, following the initial monitoring period of the Final Arsenic Rule in 2006-2008 (Nigra et al. 2020). Vital status or cause-of-death codes were determined annually by review of hospitalization records, death certificates, and/or information obtained from the National Death Index. Mortality follow up was complete in 99.8% of study participants. Cause of death was classified using the International Classification of Diseases, Ninth Revision (ICD-9) and was grouped into 4 broad categories (CVD, cancer, respiratory and infectious disease, and all other causes) by the SHS Mortality Review Committee based on standardized mortality surveillance procedures; these included discharge summary of the terminal hospital admission, medical reports, autopsy, and pathology report (if available). For CVD deaths, the ascertainment of the specific cause of death was made through a central adjudication committee (Moon et al. 2013). Detailed definitions of the criteria used by the central adjudication committee have been described previously (Moon et al. 2013; Strong Heart Study Coordinating Center 2006). Cancer mortality included death from the following cancers defined by ICD-9: esophagus and stomach (ICD-9 150-151); colon and rectum (ICD-9 153-154); liver and intrahepatic bile ducts (ICD-9 155); gallbladder and extrahepatic bile ducts (ICD-9 156); pancreas (ICD-9 157); trachea, bronchus, and lung (ICD-9 162); breast (ICD-9 174); prostate (ICD-9 185); kidney (ICD-9 189.0) skin (ICD-9 173); bladder (ICD-9 188); and lymphatic and hematopoietic tissue (ICD-9 200–208) (Garcia-Esquinas et al. 2013).

Statistical analyses

To quantify the relative hazard of mortality associated with arsenic exposure and arsenic metabolism, we used Cox proportional hazards models separately for all-cause, CVD, and cancer mortality (Cox 1972). Urine concentrations of the sum of inorganic and methylated species were modeled as quartiles and as log-transformed concentrations within an interquartile range. Arsenic metabolism (iAs%, MMA%, and DMA%) was modeled as a continuous variable within an interquartile range. The time scale for survival analysis was age in years, facilitating adjustment for this strong predictor of mortality. To handle left-truncation induced by time of enrollment and appropriately aligning risk sets on the age scale, the late entry method was conducted using individual entry time (age at baseline). To construct statistical models for three arsenic metabolism biomarkers (iAs%, MMA%, and DMA%) that are constrained to 1, we used the leave-one-out approach to address this unique

mathematic property and make interpretation meaningful. This approach has been published elsewhere (Kuo et al. 2015; Willett et al. 1997). Conventionally, prior researchers entered each arsenic metabolism biomarker into the regression model one at a time; however, this approach poses a significant challenge in interpretation as an increase in one marker could be due to the decrease in either of the other two markers (Kuo et al. 2015). In the leave-one-out approach, two biomarkers are entered at the same time, e.g., iAs% and MMA%, leaving out the third, i.e., DMA%, while holding the ΣAs constant. In the above example, the regression coefficients for iAs% estimates the hazard ratio associated with an increase in iAs% by replacing DMA% (also interpreted as decreasing DMA%) and the coefficient for MMA% estimates the hazard ratio associated with an increase in MMA% by replacing DMA% (also interpreted as decreasing DMA%).

All proportional hazards models were stratified by study site and adjusted for education level (less than high school, some high school, high school or more), smoking status (never, former, current), alcohol drinking (never, former, current), body mass index in kg/m^2 (continuous), and waist-hip ratio (continuous). We further adjusted by strong predictors of all-cause mortality including kidney function, defined by estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) equation (Levey et al. 1999), hemoglobin A1c, and fasting glucose. For cardiovascular mortality, we also adjusted by eGFR, low-density lipoprotein (LDL), diabetes, and hypertension and for cancer mortality we adjusted by eGFR, diabetes, and hypertension. We also examined whether the association between arsenic metabolism and risk of mortality varied by risk factors, including sex, smoking status, body mass index (<25, 25-30, >30 kg/m^2), and abdominal obesity (waist circumference >112 cm and >88 cm for men and women, respectively). In a sensitivity analysis we also ran the association of arsenic exposure with mortality with the full sample size, without excluding participants with arsenic species below the LOD, where levels of arsenic species below the LOD were replaced by the corresponding LOD divided by the square root of 2. All statistical analyses were performed using Stata/IC version 12 (StataCorp, College Station, Texas) and R version 3.0.0 (R foundation for Statistical Computing, Vienna, Austria).

Results

A total of 1,559 (43.3%) participants died over 51,810.3 person-years of follow-up; 484 (13.4 %) died of CVD and 281 (7.8%) died of cancer. The overall median concentration of baseline urine ΣAs was 11.2 (IQR 12.5) μg/L. Urine arsenic concentrations in participants from Arizona were 14.9 μg/L (median), 12.6 μg/L in those from North and South Dakota and 7.2 μg/L in those from Oklahoma. The overall median (IQR) for iAs%, MMA% and DMA% was 8.0 (5.4)%, 14.0 (6.8)% and 77.7 (10.7)%, respectively.

Before adjustment, participants who died during follow-up had significantly higher baseline concentrations of ΣAs but comparable relative distribution in arsenic species in urine at baseline (Table 1). An increase in IQR of baseline urine ΣAs was associated with a higher adjusted hazards ratio (HR [95%CI]) for all-cause mortality (1.28 [1.16-1.41]), CVD mortality (1.28 [1.08-1.52]), and cancer mortality (1.15 [0.92-1.44]) (Table 2 **Model 3**). The corresponding adjusted HRs for all-cause, cardiovascular, and cancer mortality without

excluding participants with arsenic below the LOD were 1.22 (1.11-1.34), 1.25 (1.07-1.48), and 1.15 (0.93-1.41), respectively, which supported the robustness of the original findings. We also found that the risk of all-cause and cardiovascular mortality increased across ΣAs quartiles 2 through 4 (p-value for trend <0.001), but not for cancer mortality (Supplementary Table 1). In a subgroup analysis, urinary ΣAs was associated with a higher risk of all-cause mortality among participants younger than 55 years and those free of diabetes or obesity (Figure 1). The associations of urinary ΣAs with CVD and cancer mortality were consistent across sub-group characteristics (Figure 1).

When modeling one arsenic metabolism biomarker at a time, an IQR increase in iAs%, MMA%, and DMA% were prospectively associated with all-cause mortality (adjusted HR (95%CI) of 0.91 (0.85 -0.97), 0.91 (0.85-0.98), and 1.12 (1.04-1.21), respectively) (Table 3, model 3). The results could lead to misunderstanding that iAs% and MMA% may be protective as the aHR was significantly below 1. However, this estimate does not allow readers to see the concomitant changes in the proportion of the other two markers making the interpretation difficult. When modeling arsenic metabolism by including two biomarkers together (leave-one-one method), the adjusted risk of mortality for an IQR range increase in iAs% was 0.97 (95% CI 0.87-1.09) when MMA% decreased and 0.93 (95% CI 0.87-0.99) when DMA% decreased. The adjusted HR (95%CI) of all-cause mortality for an IQR range increase in MMA% was 1.03 (0.90-1.19) and 0.94 (0.87-1.03) when iAs% and DMA% decreased, respectively; and for the corresponding increase in DMA%, it was 1.16 (1.01-1.33) and 1.10 (0.96-1.25) when iAs% and MMA% decreased, respectively (Table 3, model 3 and Figure 2A).

For CVD mortality, the adjusted HR (95%CI) for each IQR increase in iAs%, MMA%, and DMA% was 0.86 (0.76 -0.97), 1.05 (0.93-1.20), and 1.07 (0.94-1.21), respectively (Table 4, model 3). When modeling arsenic metabolism by including two biomarkers together, the adjusted HR (95%CI) for an IQR increase in iAs% was 0.72 (0.58-0.89) and 0.81 (0.71-0.93) when MMA% and DMA% decreased, respectively; for an IQR increase in MMA% it was 1.52 (95% CI 1.16-1.99) and 1.17 (95%CI 1.01-1.35) when iAs% and DMA% decreased, respectively; and for an IQR increase in DMA% it was 1.53 (95%CI 1.16-2.00) and 0.78 (95%CI 0.63-0.98) when iAs% and MMA% decreased, respectively (Table 4, model 3, and Figure 2B).

For cancer mortality, the adjusted HR for each IQR increase in iAs%, MMA%, and DMA% was 1.02 (95%CI 0.89 -1.17), 0.84 (95%CI 0.70-1.00), and 1.09 (95%CI 0.92-1.29), respectively (Table 5, model 3). When modeling arsenic metabolism by including two biomarkers together, the adjusted HR for an IQR increase in iAs% was 1.28 (95% CI 1.02-1.62) and 1.08 (95%CI 0.95-1.24) when MMA% and DMA% decreased, respectively; for an IQR increase in MMA% it was 0.73 (95% CI 0.55-0.98) and 0.81 (95%CI 0.67-0.97) when iAs% and DMA% decreased, respectively; and for an IQR increase in DMA% it was 0.85 (95%CI 0.65-1.12) and 1.40 (95% CI 1.04-1.87) when iAs% and MMA% decreased, respectively (Table 5, model 3, and Figure 2C). The association of patterns of arsenic metabolism with all-cause, cardiovascular, and cancer mortality is summarized in Supplementary Figure 1.

In subgroup analysis for arsenic metabolism biomarkers (Supplementary Table 2–4) , the associations of the increases in MMA% or DMA% when iAs% decreased with all-cause mortality were stronger in participants with female gender, diabetes, and obesity, but similar across all arsenic exposure categories (Supplementary Table 2). By contrast, the associations between markers of arsenic metabolism and cancer mortality were not modified across sub-group characteristics (Supplementary Table 4).

Discussion

In this US population, exposed for decades to low-to-moderate arsenic in drinking water, increasing urinary arsenic levels were associated with increased all-cause mortality and CVD mortality. A positive non-significant association was also observed between arsenic exposure and cancer mortality, which is consistent with prior reports from the Strong Heart Study (Garcia-Esquinas et al. 2013). While arsenic metabolism has been related to different health outcomes(Jansen et al. 2016; Kuo et al. 2015; Kuo et al. 2017; Wu et al. 2021), research on arsenic metabolism and mortality is scarce. We found that increasing MMA% or DMA% concomitant with decreasing iAs% (i.e., increasing methylation of inorganic As) was associated with higher all-cause mortality as well as higher CVD mortality. Increasing MMA% with relative reductions in DMA% was also associated with higher CVD mortality but not with all-cause mortality. For cancer, higher MMA% concomitant with decreasing either iAs% or DMA% was prospectively associated with lower mortality. The associations between specific patterns of arsenic metabolism biomarkers and mortality were independent of arsenic exposure levels.

The association of low-moderate arsenic exposure with all-cause mortality highlights the importance of arsenic as a contributor to disease burden and severity of disease. Our findings are consistent with those at higher levels of exposure in populations from Bangladesh (Argos et al. 2010; Rahman et al. 2019), Chile (Roh et al. 2018) and Taiwan (Chen and Wang 1990). It is worth noting that in Taiwan, there is no study reporting total mortality, only cause-specific. In these studies, while the association with all-cause mortality is largely driven by CVD, cancer mortality was also increased, although not significantly, possibly due to the smaller number of cancer deaths. Arsenic may also be related to other causes of death, but the number of outcomes for other causes were too small to small to provide adequate power to detect any associations. While few studies have evaluated the association between arsenic and total mortality, especially at low-moderate exposure levels, the body of evidence is greatest for CVD mortality (Moon et al. 2013; Moon et al. 2017). Future research efforts at low-moderate arsenic exposure in sufficiently large studies, or through pooled analyses, are needed to investigate potential multiple effect modifiers. In New Hampshire, USA, a synergistic relationship between arsenic and smoking was reported in a skin cancer case-control study (Farzan et al. 2015). By contrast, in our findings the association between arsenic and CVD mortality was stronger in non-smokers. The inconsistent findings may be related to differences in study population and statistical power.

In Taiwan’s arsenic endemic area, a significant dose-dependent association between high iAs% or low DMA% was observed with bladder cancer mortality (Chung et al. 2013). Their findings were partially consistent with ours, as increasing iAs% was also associated

with higher cancer mortality when MMA% was decreased by increasing iAs% (Figure 2). However, increasing DMA% was associated with cancer mortality when MMA% was decreased in our study. There are no studies evaluating the role of arsenic metabolism in all cancer mortality, or in all-cause or cardiovascular mortality in the context of low-moderate arsenic exposure. We found MMA% was associated with CVD mortality, regardless of which metabolism biomarkers were replaced. Increased DMA% and decreased iAs% was not only associated with increased CVD mortality but also to all-cause mortality (Figure 2). Whether or not measures to lower MMA% in the general population, such as folate supplementation (Gamble et al. 2006), can reduce the burden of CVD mortality, deserves further investigation.

The mechanism underlying the association between arsenic metabolism and mortality outcomes remains unclear though several hypotheses have been raised (Hall and Gamble 2012). One of the major hypotheses involves one carbon metabolism, which encompasses a tightly interconnected metabolic network by cycling carbon units from amino acid inputs to generate essential cellular outputs including biosynthesis, redox balance, and methylation reactions (Locasale 2013). The optimal balance between nutrition and one-carbon metabolism is critical to maintain genome stability, modulate epigenomics, and keep cellular homeostasis and detoxification (Maitra et al. 2020). Metabolic imbalance from methylation dysregulation in one-carbon metabolism has been particularly linked to the development of cancer, cardiovascular diseases, and diabetes, and could be related to the pleiotropic adverse effects of arsenic exposure (Lind et al. 2018; Zeng et al. 2020). The relative distribution of arsenic species in urine reflects both differential individual susceptibility toward arsenic exposure and differential metabolic capacity to maintain methyl balance, the fundamental driver of various downstream physiologic reactions (Steinmaus et al. 2005). Its potential to serve as metabolic signatures for mortality risk makes urinary arsenic speciation a potential tool in risk assessment. Increasing evidence has shown that nutrition (e.g. folic acid supplementation) can play a role in mitigating arsenic toxicity (Gamble et al. 2006). Our findings point to the need for experimental and clinical research to investigate the biological mechanisms and potential interventions for mitigating arsenic-related health problems, and the possibility of modifying and reducing risk through altering of arsenic metabolism.

Diabetes modifies the association between low-moderate arsenic exposure and all-cause mortality, but not for cardiovascular and cancer mortality. Moreover, among patients with diabetes, when iAs% was replaced by MMA% or DMA%, the risk of all-cause mortality was significantly augmented and the effect direction was opposite among non-diabetic patients. Effect modification of the association between arsenic metabolism and mortality by diabetes status might be the most important finding of this study. Better understanding of these differential associations for arsenic exposure and arsenic metabolism patterns by diabetes status, could be a potential target for diabetes research. An *in vitro* study reported that high cellular insulin and glucose activate homocysteine re-methylation and S-adenosylmethionine synthesis when methionine is in demand (Chiang et al. 2009). Insulin resistance in itself may alter cellular methylation balance toward higher efficiency facilitating oxidative methylation of arsenic. The impaired glutathione synthesis in patients with diabetes may further enhance the genotoxicity of pentavalent DMA (Kato et al. 2003;

Sekhar et al. 2011). However, we need to be cautious due to the limited sample size for effect modification analyses by diabetes status and the lack of reporting in other populations. Additional epidemiologic and mechanistic investigation of arsenic exposure and arsenic metabolism in mortality risk among patients with diabetes is needed.

The present study also evaluates the robustness of our findings when applying different urinary concentration corrections, including urine creatinine, specific gravity, and no correction. Generally, the statistical inferences were similar between urine creatinine and specific gravity correction for all-cause and cancer mortality, regardless of whether we looked at arsenic exposure or arsenic metabolism. For cardiovascular mortality, we found the inferences differed when comparing urine creatinine correction with specific gravity adjustment or no adjustment. Specific gravity is a measure of density and is affected by both the number of particles of solute and their molecular weight. Three common solutes – sodium chloride, glucose, and albumin can significantly contribute to the level of specific gravity (Voinescu et al. 2002). Each of them can pose significant risk for cardiovascular mortality such as high-salt diet, glucosuria, and albuminuria. Therefore, using specific gravity to correct urine dilution in evaluating cardiovascular or metabolic outcomes may lead to misclassification, overadjustment, and multi-collinearity. When not performing urine concentration correction, the resulting misclassification leads to null results regardless of the outcome of interest. These findings implied urine creatinine correction may be a better method to correct for urine dilution when evaluating the effect of arsenic exposure for cardiovascular or metabolic outcomes, which is consistent with a recent review (Hsieh et al. 2019).

Our study systematically examined the relationship between arsenic metabolism and all-cause, cancer and CVD mortality using data from a population-based cohort at low-moderate levels of exposure. The SHS cohort represented an ideal cohort with a standardized protocol to ascertain mortality data over a 20-year follow-up and high-quality laboratory data of concentrations of urine arsenic species. In addition to the strength of the cohort, our modeling of arsenic metabolism accounted for the inter-relatedness of the proportion of arsenic species (iAs%, MMA%, DMA%). Despite the single measurement at baseline, the urine concentrations of arsenic species in the Strong Heart Study population were stable over a 10-year follow up (between 1989-1991 and 1998-1999), and have likely remained stable throughout the follow-up as the implementation of the arsenic MCL (maximum contaminant level) generally took place after the initial monitoring period for the Final Arsenic Rule in 2006-2008, in the US at large and also in the SHS communities (Navas-Acien et al. 2009; Nigra et al. 2020). In addition, no household-level water arsenic data is available in the SHS, although general information is known for community water arsenic, these data are not useful to evaluate water arsenic levels by outcome status. It is also possible that our models were over-adjusted for variables possibly in the causal pathway (e.g., HbA1c and fasting glucose in all-cause mortality) or that our models missed unknown or unmeasured confounders. Nonetheless, model 1 and 2 of the risk association analysis provided similar statistical inferences. Finally, given the observational nature of this study, whether the association between arsenic metabolism and all-cause and cause-specific mortality reflects cause and effect is unknown.

Conclusion

This study evaluated the association of low-moderate arsenic exposure and the relative distribution of arsenic species in urine with all-cause, CVD, and cancer mortality within the same large, prospective cohort. Increasing MMA%, regardless of whether it was replaced by either decreasing iAs% or DMA%, was associated with higher CVD mortality but lower cancer mortality. DMA% was positively associated with CVD mortality when replacing iAs% and with cancer mortality when replacing MMA%. Using arsenic metabolism biomarkers to determine individual susceptibility and risk of mortality from arsenic exposure could play a critical role in future arsenic risk assessment. Additional experimental and epidemiological evidence are needed to understand the biological mechanisms and clinical implications of arsenic metabolism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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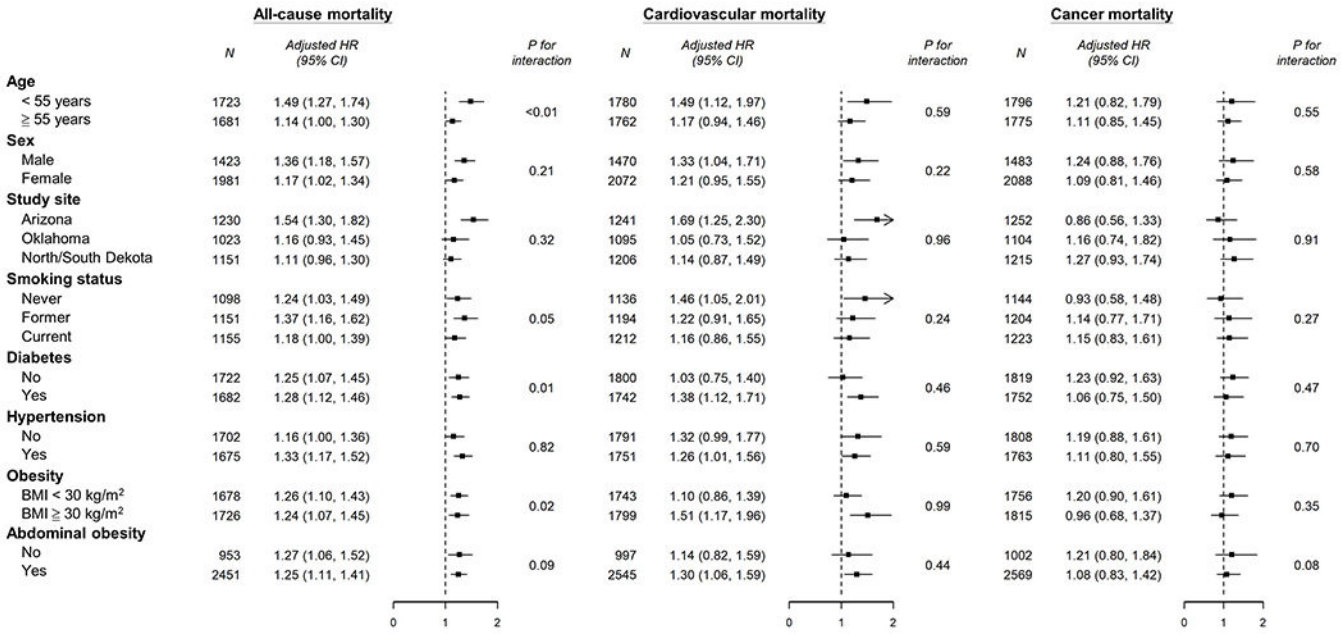

Figure 1.

Subgroup analysis for associations between inorganic arsenic exposure (ΣAs) and outcomes of all-cause, cardiovascular, and cancer mortality according to baseline characteristics.

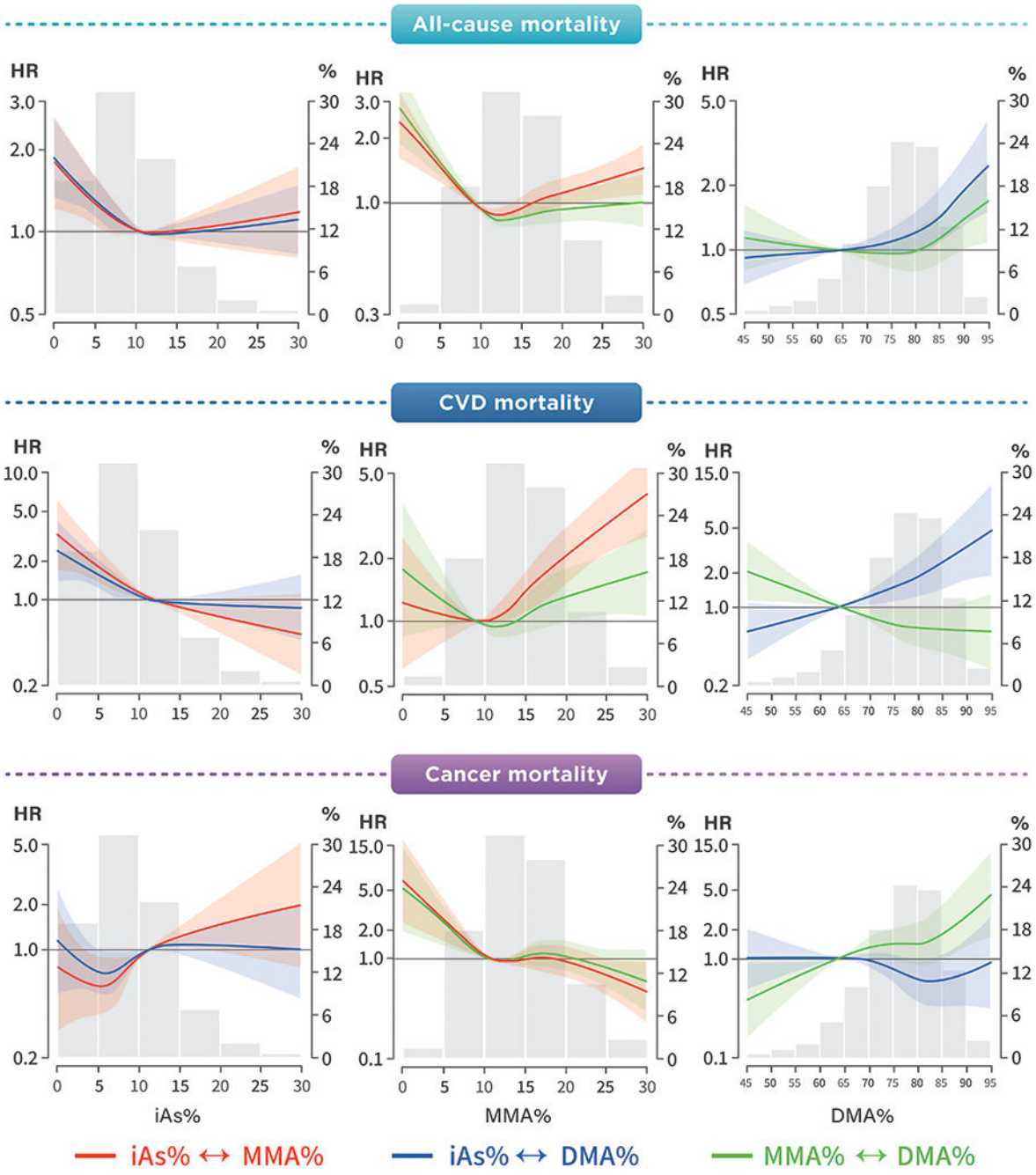

Figure 2. Hazard ratios for all-cause (upper panel), cardiovascular (middle panel), and cancer mortality (lower panel) by biomarkers of arsenic metabolism.

Solid lines and shaded area represent adjusted hazard ratios based on restricted quadratic splines with 95% confidence interval using knots at the 10th, 50th, and 90th percentiles. The solid line represents the hazard ratio for iAs% when it replaces MMA% (red line) and DMA% (blue line) in the left panel, the hazard ratio for MMA% when it replaces iAs% (red line) and DMA% (green line) in the middle panel and the hazard ratio for DMA% when it replaces iAs% (blue line) and MMA% (green line).

Table 1.

Characteristics of Strong Heart Study participants at baseline (1989-1991) [a]

	Survivors n=2,041, 56.7%	**Deaths n=1559, 43.3%**	***p*-value**
Age, year	52.7 (48.3-58.7)	58.3 (51.4-65.5)	<0.01
Male	756 (37.0)	740 (47.5)	<0.01
Education (yrs)			<0.01
No high school	333 (16.3)	484 (31.1)	
Some high school	456 (22.3)	429 (27.5)	
High school or more	1252 (61.3)	646 (41.4)	
Smoking (%)			0.44
Never	669 (32.8)	485 (31.1)	
Former	691 (33.9)	525 (33.7)	
Current	681 (33.4)	549 (35.2)	
Alcohol (%)			0.25
Never	310 (15.2)	255 (16.4)	
Former	868 (42.5)	621 (39.8)	
Current	863 (42.3)	683 (43.8)	
Body mass index, kg/m^2	30.4 (27.1-34.6)	30.0 (26.0-33.9)	<0.01
Waist-hip ratio	0.95 (0.91-0.98)	0.96 (0.93-1)	<0.01
Waist circumference, cm	104 (96-115)	104 (95-114)	0.81
Percent body fat	37.8 (30.4-43.9)	34.5 (28.2-42)	<0.01
eGFR, ml/min/1.73m^2	81.9 (72.7-94.1)	80.7 (68.0-93.7)	<0.01
Hypertension	616 (30.2)	754 (48.4)	<0.01
Diabetes mellitus	807 (39.5)	959 (61.5)	<0.01
Fasting glucose, mg/dL	110 (98-142)	129 (103-218)	<0.01
HbA1c, % [b]	5.4 (4.9-6.6)	6.1 (5.1-9.2)	<0.01
Urine arsenic exposure			
ΣAs [c], µg/g	8.9 (5.6-14.2)	11.9 (7.1-18.4)	<0.01
iAs, µg/g	0.7 (0.4-1.3)	0.9 (0.4-1.7)	<0.01
MMA, µg/g	1.2 (0.7-2.0)	1.6 (0.9-2.7)	<0.01

	Survivors n=2,041, 56.7%	Deaths n=1559, 43.3%	*p*-value
DMA, μg/g	6.7 (4.2-11.0)	9.1 (5.4-14.2)	<0.01
Arsenic metabolism			
iAs%	8.0 (5.6-11.0)	7.9 (5.6-11.0)	0.37
MMA%	13.9 (11.0 -17.4)	14.1 (10.6-17.8)	0.59
DMA%	77.7 (72.0-82.5)	77.8 (71.7-82.9)	0.40

Abbreviations: iAs, inorganic arsenic (arsenite and arsenate); DMA, dimethylarsinate; MMA, monomethylarsonate; eGFR, estimated glomerular filtration rate.

[a] Data are n (%) or median (interquartile range)

[b] Among 1,922 participants with available HbA1c data

[c] ΣAs=iAs + methylated arsenic

Table 2.

Hazard ratio (95% confidence interval) for different mortality outcomes per interquartile range in urine concentrations of inorganic arsenic (iAs), monomethylarsonate (MMA), dimethylarsinate (DMA) and the sum of iAs, MMA and DMA (μg/L).

Arsenic (interquartile range)	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
Urinary dilution control	**Urine creatinine**	**Urine creatinine**	**Urine creatinine**	**Specific gravity**	**None**
All-cause mortality	1559/2041	1559/2041	1482/3404	1482/3404	1482/3404
iAs (0.4-1.7 μg/L)	1.08 (0.99-1.18)	1.03 (0.94-1.13)	1.06 (0.97-1.17)	1.07 (0.99-1.17)	0.97 (0.89-1.05)
MMA (0.8-2.8 μg/L)	1.15 (1.05-1.26)	1.12 (1.02-1.24)	1.11 (1.00-1.22)	1.08 (0.99-1.17)	0.96 (0.89-1.04)
DMA (5.1-14.5 μg/L)	1.35 (1.23-1.47)	1.31 (1.19-1.43)	1.29 (1.18-1.42)	1.19 (1.11-1.29)	1.04 (0.97-1.12)
ΣAs (6.6-19.1 μg/L)	1.33 (1.21-1.45)	1.28 (1.17-1.40)	1.28 (1.16-1.41)	1.18 (1.09-1.28)	1.03 (0.96-1.12)
Cardiovascular disease mortality	484/3116	484/3116	480/3062	480/3062	480/3062
iAs (0.4-1.7 μg/L)	0.97 (0.83-1.13)	0.94 (0.80-1.11)	1.02 (0.86-1.20)	0.93 (0.81-1.08)	0.88 (0.75-1.02)
MMA (0.8-2.8 μg/L)	1.20 (1.02-1.42)	1.21 (1.02-1.43)	1.25 (1.05-1.49)	1.02 (0.89-1.17)	0.96 (0.83-1.10)
DMA (5.1-14.5 μg/L)	1.34 (1.15-1.57)	1.36 (1.16-1.60)	1.28 (1.08-1.51)	1.02 (0.89-1.16)	0.96 (0.84-1.09)
ΣAs (6.6-19.1 μg/L)	1.31 (1.11-1.55)	1.29 (1.09-1.52)	1.28 (1.08-1.52)	1.02 (0.89-1.17)	0.95 (0.83-1.09)
Cancer mortality	281/3319	281/3319	281/3290	281/3290	281/3290
iAs (0.4-1.7 μg/L)	1.15 (0.94-1.41)	1.12 (0.91-1.38)	1.13 (0.91-1.39)	1.14 (0.95-1.37)	1.03 (0.85-1.25)
MMA (0.8-2.8 μg/L)	1.02 (0.82-1.27)	0.98 (0.79-1.23)	0.99 (0.79-1.24)	1.01 (0.84-1.21)	0.91 (0.76-1.09)
DMA (5.1-14.5 μg/L)	1.17 (0.95-1.44)	1.18 (0.95-1.46)	1.17 (0.95-1.45)	1.14 (0.96-1.36)	1.00 (0.85-1.18)
ΣAs (6.6-19.1 μg/L)	1.16 (0.94-1.44)	1.16 (0.93-1.44)	1.15 (0.92-1.44)	1.13 (0.95-1.35)	0.99 (0.84-1.18)

Model 1: Stratified by study center and adjusted for age (age as time metric and age at baseline were treated as staggered entries) and urine creatinine (log-transformed), sex, and education

Model 2: Further adjusted for smoking, alcohol drinking, body mass index and waist-hip ratio

Model 3: for all-cause mortality: Further adjusted for estimated glomerular filtration rate, baseline hemoglobin A1c and fasting glucose level.

for cardiovascular disease mortality: Further adjusted for estimated glomerular filtration rate, LDL, diabetes(yes/no), and hypertension(yes/no)

for cancer mortality: Further adjusted for estimated glomerular filtration rate, diabetes(yes/no), and hypertension(yes/no)

Model 4: Urine creatinine level in model 3 was replaced by urine specific gravity

Model 5: Model 3 without urine creatinine

Table 3.

Hazard ratio (95% confidence interval) for **all-cause mortality** per interquartile range in arsenic metabolism biomarkers (inorganic arsenic% [iAs%], monomethylarsonate% [MMA%], and dimethylarsinate %[DMA%]). As the three biomarkers equal 100%, models entered two arsenic metabolism biomarkers at a time.

Arsenic metabolism (interquartile range)	Model 1	Model 2	Model 3 (N=3404)	Model 4 (N=3404)	Model 5 (N=3404)
Urinary dilution control	**Urine creatinine**	**Urine creatinine**	**Urine creatinine**	**Specific gravity**	**None**
One metabolism biomarker in each model					
iAs%	0.89 (0.83-0.95)	0.87 (0.82-0.93)	0.91 (0.85-0.97)	0.95 (0.89-1.01)	0.96 (0.90-1.02)
MMA%	0.91 (0.85-0.98)	0.90 (0.84-0.97)	0.91 (0.85-0.98)	0.91 (0.84-0.98)	0.90 (0.84-0.97)
DMA%	1.14 (1.07-1.22)	1.17(1.09-1.25)	1.12 (1.04-1.21)	1.09 (1.02-1.18)	1.09 (1.01-1.17)
Two metabolism biomarkers in each model					
iAs% substituted by:					
MMA% (10.8-17.6)	1.08 (0.94-1.24)	1.10 (0.95-1.26)	1.03 (0.90-1.19)	0.95 (0.83-1.09)	0.91 (0.80-1.05)
DMA% (71.9-82.6)	1.22 (1.07-1.40)	1.26 (1.10-1.45)	1.16 (1.01-1.33)	1.05 (0.92-1.20)	1.01 (0.89-1.16)
MMA% substituted by:					
iAs% (5.6-11.0)	0.94 (0.84-1.05)	0.93 (0.83-1.04)	0.97 (0.87-1.09)	1.04 (0.93-1.16)	1.07 (0.96-1.20)
DMA% (71.9-82.6)	1.08 (0.95-1.22)	1.09 (0.96-1.24)	1.10 (0.96-1.25)	1.14 (1.00-1.30)	1.17 (1.03-1.33)
DMA% substituted by:					
iAs% (5.6-11.0)	0.90 (0.84-0.97)	0.89 (0.83-0.95)	0.93 (0.87-0.99)	0.98 (0.91-1.04)	0.99 (0.93-1.06)
MMA% (10.8-17.6)	0.95 (0.88-1.03)	0.95 (0.87-1.03)	0.94 (0.87-1.03)	0.92 (0.85-1.00)	0.91 (0.83-0.98)

Model 1: Stratified by study center, adjusted for age (age as time metric and age at baseline were treated as staggered entries), the sum of inorganic arsenic and methylated arsenic concentrations (log-transformed), urine creatinine levels (log-transformed), sex, and education

Model 2: Further adjusted for smoking, alcohol drinking, body mass index and waist-hip ratio.

Model 3: Further adjusted for estimated glomerular filtration rate, baseline hemoglobin A1c and fasting glucose level.

Model 4: Urine creatinine level was replaced by urine specific gravity in model 3.

Model 5: Model 3 without urine creatinine

Table 4.

Hazard ratio (95% confidence interval) for **cardiovascular mortality** per interquartile range in arsenic metabolism biomarkers (inorganic arsenic% [iAs%], monomethylarsonate% [MMA%] and dimethylarsinate% [DMA%]). As the three biomarkers equal 100%, models entered two arsenic metabolism biomarkers at a time.

Arsenic metabolism (interquartile range)	**Model 1**	**Model 2**	**Model 3 (N=3542)**	**Model 4 (N=3542)**	**Model 5 (N=3542)**
Urinary dilution control	**Urine creatinine**	**Urine creatinine**	**Urine creatinine**	**Specific gravity**	**None**
One metabolism biomarker in each model					
iAs%	0.79 (0.70-0.89)	0.78 (0.69-0.88)	0.86 (0.76-0.97)	0.94 (0.83-1.05)	0.93 (0.83-1.05)
MMA%	0.97 (0.86-1.10)	1.00 (0.88-1.14)	1.05 (0.93-1.20)	1.05 (0.92-1.19)	1.04 (0.92-1.19)
DMA%	1.18 (1.04-1.33)	1.17 (1.03-1.33)	1.07 (0.94-1.21)	1.02 (0.89-1.15)	1.02 (0.90-1.16)
Two metabolism biomarkers in each model					
iAs% substituted by:					
MMA% (10.8-17.6)	1.57 (1.22-2.04)	1.67 (1.29-2.18)	1.52 (1.16-1.99)	1.26 (0.97-1.64)	1.25 (0.96-1.63)
DMA% (71.9-82.6)	1.75 (1.34-2.29)	1.82 (1.39-2.39)	1.53 (1.16-2.00)	1.23 (0.95-1.61)	1.23 (0.95-1.60)
MMA% substituted by:					
iAs% (5.6-11.0)	0.70 (0.57-0.86)	0.66 (0.54-0.82)	0.72 (0.58-0.89)	0.83 (0.67-1.03)	0.86 (0.69-1.08)
DMA% (71.9-82.6)	0.85 (0.69-1.06)	0.81 (0.65-1.01)	0.78 (0.63-0.98)	0.86 (0.68-1.08)	0.84 (0.68-1.03)
DMA% substituted by:					
iAs% (5.6-11.0)	0.75 (0.66-0.86)	0.74 (0.65-0.85)	0.81 (0.71-0.93)	0.90 (0.79-1.03)	0.90 (0.79-1.03)
MMA% (10.8-17.6)	1.11 (0.96-1.27)	1.15 (1.00-1.32)	1.17 (1.01-1.35)	1.10 (0.95-1.28)	1.10 (0.95-1.27)

Model 1: Stratified by study center, adjusted for age (age as time metric and age at baseline were treated as staggered entries), the sum of inorganic arsenic and methylated arsenic concentrations (log-transformed), urine creatinine levels (log-transformed), sex, and education

Model 2: Further adjusted for smoking, alcohol drinking, body mass index and waist-hip ratio.

Model 3: Further adjusted for estimated glomerular filtration rate, low-density lipoprotein, diabetes(yes/no), and hypertension(yes/no)

Model 4: Urine creatinine level was replaced by urine specific gravity in model 3.

Model 5: Model 3 without urine creatinine

Table 5.

Hazard ratio (95% confidence interval) for **cancer mortality** per interquartile range in arsenic metabolism biomarkers (inorganic arsenic% [iAs%], monomethylarsonate% [MMA%] and dimethylarsinate% [DMA%]). As the three biomarkers equal 100%, models entered two arsenic metabolism biomarkers at a time.

Arsenic metabolism (interquartile range)	**Model 1**	**Model 2**	**Model 3 (N=3571)**	**Model 4 (N=3571)**	**Model 5 (N=3571)**
Urinary dilution control	**Urine creatinine**	**Urine creatinine**	**Urine creatinine**	**Specific gravity**	**None**
One metabolism biomarker in each model					
iAs%	1.04 (0.92-1.19)	1.01 (0.88-1.16)	1.02 (0.89-1.17)	1.05 (0.92-1.20)	1.06 (0.93-1.21)
MMA%	0.88 (0.75-1.04)	0.83 (0.70-0.99)	0.84 (0.70-1.00)	0.84 (0.70-1.00)	0.83 (0.70-0.99)
DMA%	1.05 (0.89-1.23)	1.10 (0.93-1.31)	1.09 (0.92-1.29)	1.07 (0.90-1.26)	1.07 (0.90-1.26)
Two metabolism biomarkers in each model					
iAs% substituted by:					
MMA% (10.8-17.6)	0.76 (0.57-1.00)	0.73 (0.55-0.98)	0.73 (0.55-0.98)	0.70 (0.53-0.92)	0.68 (0.52-0.90)
DMA% (71.9-82.6)	0.84 (0.65-1.09)	0.86 (0.66-1.13)	0.85 (0.65-1.12)	0.81 (0.63-1.05)	0.79 (0.62-1.02)
MMA% substituted by:					
iAs% (5.6-11.0)	1.25 (1.00-1.55)	1.28 (1.02-1.61)	1.28 (1.02-1.62)	1.33 (1.07-1.66)	1.35 (1.09-1.68)
DMA% (71.9-82.6)	1.30 (0.99-1.72)	1.41 (1.06-1.89)	1.40 (1.04-1.87)	1.43 (1.07-1.91)	1.45 (1.09-1.94)
DMA% substituted by:					
iAs% (5.6-11.0)	1.09 (0.96-1.24)	1.08 (0.94-1.23)	1.08 (0.95-1.24)	1.11 (0.98-1.26)	1.12 (0.99-1.27)
MMA% (10.8-17.6)	0.85 (0.71-1.01)	0.80 (0.67-0.97)	0.81 (0.67-0.97)	0.80 (0.66-0.96)	0.79 (0.66-0.95)

Model 1: Stratified by study center, adjusted for age (age as time metric and age at baseline were treated as staggered entries), the sum of inorganic arsenic and methylated arsenic concentrations (log-transformed), urine creatinine levels (log-transformed), sex, and education

Model 2: Further adjusted for smoking, alcohol drinking, body mass index and waist-hip ratio.

Model 3: Further adjusted for estimated glomerular filtration rate, diabetes(yes/no), and hypertension(yes/no)

Model 4: Urine creatinine level was replaced by urine specific gravity in model 3.

Model 5: Model 3 without urine creatinine