THE ROLE OF ARSENIC METABOLISM IN MORTALITY, DIABETES, AND KIDNEY DISEASE

by

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Abstract

Experimental and epidemiological evidence supports the role of chronic arsenic exposure in a broad scope of adverse health effects at a wide range of exposure levels. However, little is known regarding arsenic metabolism and health risk. The objective of this dissertation was to investigate the role of arsenic metabolism in mortality, diabetes, and kidney disease.

First, we conducted a systematic review of the epidemiologic evidence examining the relation between arsenic metabolism and cancer, cardiovascular diseases, and adiposity. We identified 12 eligible studies for cancer, 9 for cardiovascular diseases, and 7 for adiposity. The higher proportion of monomethylarsonate [MMA%] in the urine tended to be associated with cancer and cardiovascular disease risk, whereas the lower MMA% tended to associated with an increase in adiposity. However, rather heterogeneous statistical approaches and limited prospective evidence prevented a conclusive inference from this review. In variability analysis, the range of between-population variation in MMA% is relatively narrow compared to the proportion of inorganic arsenic [iAs%] and dimethylarsinate [DMA%] in urine.

Second, we measured arsenic metabolism defined by relative proportions of inorganic arsenic, MMA and DMA over their sum in the baseline urine of Strong

Heart Study participants aged 45-74 years to evaluate the role of arsenic metabolism in all-cause, cardiovascular disease and cancer mortality. The adjusted hazard ratio of all-cause mortality for an interquartile increase in DMA% was 1.16 (95% CI 1.01-1.33) when it substituted iAs% whereas MMA% did not explain the risk of all-cause mortality. For cardiovascular mortality, the adjusted hazard ratio for an interquartile change increase in MMA% was 1.52 (1.16-1.99) and 1.17 (1.01-1.35) when it substituted iAs% and DMA%, respectively. For cancer mortality, the adjusted hazard ratio for an interquartile increase in MMA% was 0.73 (0.55-0.98) and 0.81 (0.67-0.97) when it substituted iAs% and DMA%, respectively.

Third, we examine the prospective association between arsenic metabolism and diabetes in the Strong Heart Study. The adjusted hazard ratios of diabetes for an interquartile range increase in MMA% was 0.69 (95% CI 0.52-0.90) and 0.76 (0.65-0.89) when it was substituted for iAs% and DMA%, respectively. The association between arsenic metabolism and diabetes was similar by age, sex, study site, obesity, and the sum of inorganic and methylated arsenic concentrations.

Fourth, we evaluated the role of arsenic metabolism in the development of chronic kidney disease among Strong Heart Study participants without baseline kidney disease. Incident kidney disease was defined by estimated glomerular filtration rate(eGFR) <60 ml/min/1.73m^2 with a drop in eGFR $\geq 25\%$. The adjusted hazard

ratio for an interquartile range increase in MMA% was 1.76 (95% CI 1.26-2.47) and 1.22 (1.02-1.45) when it was substituted for iAs% and DMA%, respectively. And when an interquartile range increase in DMA% with a corresponding decrease in iAs%, the adjusted hazard ratio was 1.83 (95% CI 1.29-2.61).

In conclusion, arsenic metabolism was significantly associated with the risk of mortality, diabetes, and kidney disease and the associations were independent of total chronic arsenic exposure. Our results support that urine biomarkers of arsenic metabolism may reflect individual susceptibility to arsenic-related health effects and provide a novel perspective on the dynamic modeling of arsenic metabolism. In addition to replicating these finding across diverse populations and geographical areas to advance risk assessment and risk management of arsenic, future research needs to evaluate mechanisms for the connection between arsenic metabolism and health outcomes.

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To my parents, for their endless love, care, and unconditional encouragement,

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"If I have seen further, it is by standing on the shoulders of giants"

- Sir Isaac Newton (1676)

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Abbreviations

10f-THF	10-Formyltetrahydrofolate
10f0DHF	10-Formyldihydrofolate
5fTHF	5-Formyltetrahydrofolate (leucovorin)
5mTHF	5-Methyltetrahydrofolate
AICAR	P-ribosyl-5-amino-4-imidazole carboxamide
AICART	Aminoimidazolecarboxamide ribonucleotide transferase
As[III]	Arsenite
As[V]	Arsenate
$As[III](GS)_3$	Arsenotriglutathione
AS3MT	Arsenic(+III Oxidation State) Methyltransferase
ATG	Arsenic triglutathione
BET, Bet	Betaine
BHMT	Betaine-homocysteine methyltransferase
CBS	Cystathionine β-synthase
CH:NHTHF	5-Formiminotetrahydrofolate
CH=THF	5–10-Methenyltetrahydrofolate
CH2-THF	5–10-Methylenetetrahydrofolate
CTGL	γ-Cystathionase
Cys	Cysteine
Cyst	Cystathionine
DHF	Dihydrofolate
DHFR	Dihydrofolate reductase
DHFS	Dihydrofolate synthase

DHPR	Dihydropteridine reductase
DMA[III]	Dimethylarsinite
DMA[III](GS)	Dimethylarsenoglutathione
DMA[V]	Dimethylarsinate
DMAG	Dimethylarsinic glutathione
DMG	Dimethylglycine
DMGD	Dimethylglycine dehydrogenase
DNMT	DNA-methyltransferase
dTMP	Deoxythymidine monophophate
dUMP	Deoxyuridine monophophate
FOCM	Folate-mediated one-carbon metabolism
FR-RFC	Folate receptor – reduced folate carrier
FTD	10-Formyltetrahydrofolate dehydrogenase
FTD	10-Formyltetrahydrofolate dehydrogenase
FTS	10-Formyltetrahydrofolate synthase
GAR	Glycinamide ribonucleotide
GCS	γ-Glutamylcysteine synthetase
GDC	Glycine decarboxylase (glycine cleavage system)
Glut	Glutamate
Glut-Cys	Glutamyl-cysteine
Gly	Glycine
GNMT	Glycine N-methyltransferase
GPX	Glutathione peroxidase
GR	Glutathione reductase

GS	Glutathione synthetase
GSH	Reduced glutathione
GSSG	Oxidized glutathione disulfide
$H_2C=O$	Formaldehyde
H_2O_2	Hydrogen peroxide
HCOOH	Formate
Hcy	Homocysteine
MADG	Monomethyarsonic diglutathione
MAT-I	Methionine adenosyl transferase I
MAT-II	Methionine adenosyl transferase II
MAT-III	Methionine adenosyl transferase III
Met	Methionine
MMA[III]	Monomethylarsonite
MMA[III]$(GS)_2$	Monomethylarsenodiglutathione
MMA[V]	Monomethylarsonate
MS	Methionine synthase
MTCH	5,10-Methenyltetrahydrofolate cyclohydrolase
MTD	5,10-Methylenetetrahydrofolate dehydrogenase
MTHFR	5,10-Methylenetetrahydrofolate reductase
MTS	5,10-Methenyltetrahydrofolate synthetase
NADPH	Nicotinamide adenine dinucleotide phosphate
NE	non-enzymatic conversion
PGT	Phosphoribosyl glycinamidetransformylase
SAH	S-adenosylhomocysteine

SAHH	S-adenosylhomocysteine hydrolase
SAM	S-adenosylmethionine
Sarc	Sarcosine
SDH	Sarcosine dehydrogenase
Ser	Serine
SHMT	Serinehydroxymethyltransferase
TS	Thymidylate synthase
THF	Tetrahydrofolate

INTRODUCTION

Specific aims

Arsenic metabolism refers to the process of how arsenic is methylated and transformed into arsenic metabolites in the human body. There is a substantial inter-individual variation in arsenic methylation efficiency.[1, 2] The biological meaning underlying this variation, however, remains poorly understood. Increasing epidemiologic evidence supports ***arsenic metabolism*** as an important determinant of individual susceptibility to the adverse effects of inorganic exposure including cancers[3], cardiovascular diseases[4], and diabetes mellitus (DM).[5] Most studies were cross-sectional and case-control in design with relatively small samples and high levels of arsenic exposure. For US populations, arsenic exposure is pervasive through drinking water and foods especially in small rural communities affected by low-to-moderate arsenic levels in drinking water .[6, 7] The associations between low-moderate arsenic exposure, cardiovascular disease, and type 2 DM has been recently reported in both cross-sectional and prospective studies.[8-11] Yet, little is known about the impact of low-moderate arsenic exposure in ***all-cause mortality and kidney diseases*** and large prospective evidence examining the association between arsenic metabolism and non-cancer outcomes at levels of arsenic exposure relevant to US populations is lacking.

The **first objective** of this project is to evaluate the role of arsenic metabolism in ***mortality from all-cause, cardiovascular disease, and cancer*** among participants in the **Strong Heart Study (SHS)**. Starting in 1989, the SHS recruited resident members in American-Indians (age 45-74 years) from communities in Arizona, Oklahoma and the North and South Dakotas to investigate the development of obesity, diabetes and cardiovascular diseases.[12] The **second objective** is to estimate the association of arsenic metabolism and arsenic exposure on incident diabetes. The increasing prevalence of type 2 DM poses a major public health challenge and diabetes is strongly associated with all-cause mortality, cardiovascular morbidities, and ends stage renal disease.[13, 14] Although large evidence supports the role of arsenic exposure in the development of diabetes,[15] the debate about the causality of the association remains unsettled, especially at low-moderate levels of arsenic exposure.[16] Especially, very few studies have evaluated the role of arsenic metabolism in the development of diabetes. The **third objective** is to evaluate the role of arsenic in the development of chronic kidney diseases (CKD). Chronic kidney disease is a significant global health issue with prevalence 8-16% worldwide and the burden of CKD is also rising due to multiple organ complications and increased all-cause and cardiovascular mortality.[17-19] Growing evidence links inorganic arsenic exposure to

diverse renal injuries including proteinuria[20], chronic kidney disease[21], and proximal tubular dysfunction .[22] Characterizing the potential role of arsenic exposure and arsenic metabolism provides a novel perspective on CKD prevention. Given the widespread exposure to arsenic and the high mortality rate and high incident diabetes in American Indians, our study can inform integrated risk assessment of arsenic toxicity and arsenic metabolism and advance the prevention of diabetes and CKD.

The **specific aims of this dissertation** are the following:

1. **To conduct a systematic review of the epidemiologic association between arsenic metabolism and cancer, cardiovascular disease and adiposity/diabetic phenotypes.** While an increasing number of studies have evaluated the association between arsenic metabolism and disease, no systematic review has been previously conducted on this topic.
2. **To evaluate the association of long-term arsenic exposure and metabolism with all-cause, cardiovascular, and cancer mortality.** Arsenic exposure was assessed based on the sum of inorganic, monomethylated (MMA) and dimethylated (DMA) arsenic species in urine. Arsenic metabolism was assessed based on the relative proportion of inorganic arsenic, MMA and DMA over their sum. We have previously confirmed the long-term stability of urine arsenic concentrations and methylation patterns over 10 years in this population,

supporting the use of a single urine sample to assess arsenic internal dose and metabolism in this study.[23] Causes of death were determined by the SHS Mortality Review Committee based on the standardized mortality surveillance procedures, including discharge summary of the terminal hospital admission, medical reports, autopsy, and pathology report if available[8].

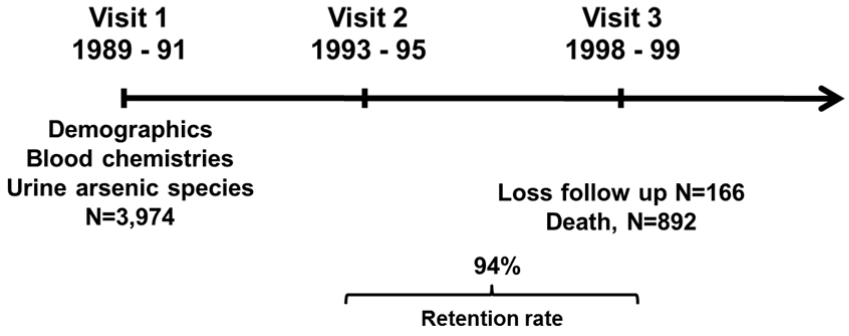
3. **To evaluate the association of long-term arsenic exposure and metabolism with the incidence of type 2 diabetes.** We hypothesized that inorganic arsenic exposure is associated with increased risk of incident diabetes as arsenic has been linked to various diabetogenic mechanisms including beta-cell dysfunction and systemic insulin resistance.[24-26] We also hypothesized that different arsenic methylation capacity predisposes the individual to incident diabetes. We used the measures of arsenic exposure and metabolism described in aim 1 to evaluate the prospective association between arsenic exposure, arsenic metabolism and incident diabetes. Diabetes will be defined according to the latest World Health Organization (WHO) guideline using measures currently available for all SHS participants at baseline and up to 2 follow-up visits.[27]

4. **To evaluate the association of long-term arsenic exposure and metabolism with incident chronic kidney diseases.** We hypothesized that vascular inflammation and endothelial dysfunction induced by inorganic arsenic may be

associated with the development of chronic kidney diseases[20]. We also hypothesized that differential arsenic biotransformation capacity links to different incident CKD risk. We used the measures of arsenic exposure and metabolism described in aim 1 to evaluate the incidence of CKD among participants with baseline estimated glomerular filtration rate higher than 60 ml/min/1.73m^2. CKD was defined by four commonly adopted definitions in cohort studies including 1) an eGFR less than 60 ml/min/1.73m^2; 2) an eGFR less than 60 ml/min/1.73m^2 and a drop in eGFR of at least 25%; 3) an eGFR less than 60 ml/min/1.73m^2 and a drop in eGFR of at least 25% and with macroalbuminuria (urine albumin-creatinine ratio ≥ 300 mg/g creatinine); 4) doubling serum creatinine levels or progression to end-stage renal disease (ESRD)[28, 29]. Associations were adjusted for risk factors of cardiovascular diseases including hypertension and diabetes.

The SHS is the largest population-based study of diabetes and cardiometabolic diseases in American Indians (Figure 1)[12, 30]. Exposure to a wide range of low-moderate inorganic arsenic, a high mortality rate, a high burden of diabetes and obesity and a low seafood exposure make an excellent opportunity to study the adverse health effects of inorganic arsenic in this population with the potential to generalize study results to the general U.S. population which also has low-moderate arsenic exposure and a high burden of obesity.

Figure 1. SHS clinic visits, follow-up and data to be used in the proposed study



Overview of arsenic metabolism

Biotransformation of arsenic in human

Inorganic arsenic is metabolized in the human body. After absorption, the inorganic forms (arsenate and arsenite) are methylated into monomethylarsonate [MMA] and dimethylarsinate [DMA]) mainly in the liver and excreted in the urine through kidney (Figure 2).[31, 32] Among many proposed arsenic biotransformation pathways[33-38], two inorganic arsenic metabolic pathways have been commonly described: 1) Classical oxidative methylation pathway (Challenger pathway) involving sequential steps from As[V]) → arsenite (As[III]) → monomethylarsonate (MMA[V]) → monomethylarsonite (MMA[III]) → dimethylarsinate (DMA[V]) → dimethylarsinite (DMA[III])[33, 34, 39]; 2) Alternate reductive methylation pathway (Hayakawa pathway) involving the conjugation between arsenite(As[III]) and glutathione(GSH) from transsulfuration pathway and a subsequent methylation from

arsenotriglutathione(As[III](GS)$_3$) → monomethylarsenodiglutathione (MMA[III](GS)$_2$) → dimethylarsenoglutathione (DMA[III](GS)).[35] The oxidation of MMA[III](GS)$_2$ and DMA[III](GS) to MMA[V] and DMA[V] could be separated and does not have to be sequential.[35, 39] Overall, the exact biotransformation pathway of arsenic remains not fully understood after about 60 years of research in this domain, Cullen et al. recently summarized various perspectives on arsenic biomethylation and concluded that the Challenger pathway remains the most rational possibility.[38] The relative toxicities of the arsenic metabolites has been proposed based on genotoxicity largely from cell culture studies in the following rank: MMA[III] > DMA[III] > As[III] > As[V] > MMA[V] > DMA[V].[40] However, the feasibility to generalize this toxicity rank to the human population is unknown.

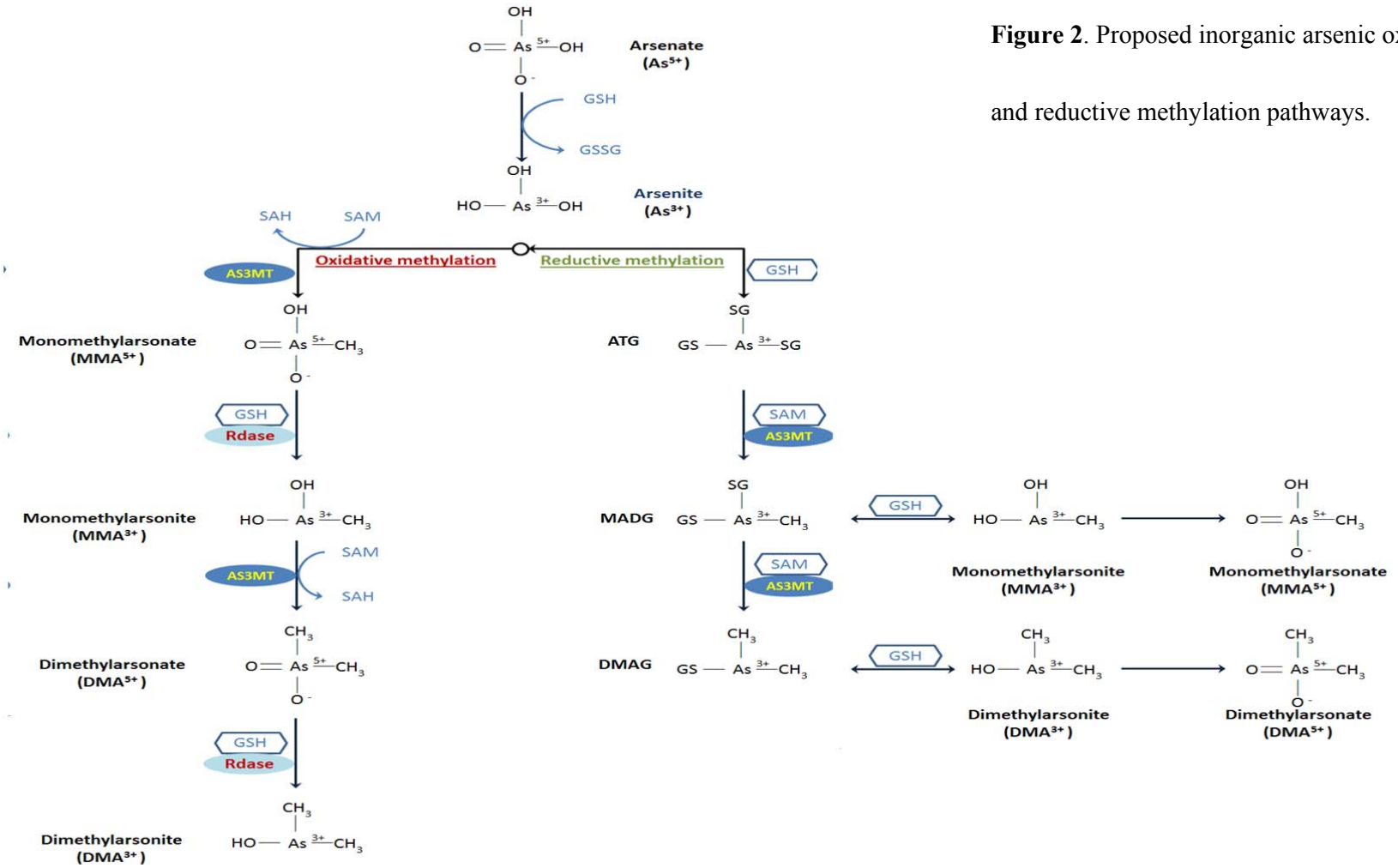


Figure 2. Proposed inorganic arsenic oxidative and reductive methylation pathways.

Determinants of arsenic metabolism

In humans, the average distribution of arsenic metabolites in urine is 10-30% inorganic arsenic, 10-20% MMA and 60-80% DMA,[41-43] with both substantial intra-individual and inter-individual variation.[44-47] Similar to other biochemical methylation processes, the contributions of both genetic and environmental factors to the inter-individual variability of arsenic metabolism are widely acknowledged.[46] Probably, the most well-known genetic variation involving the arsenic methylation efficiency is the genetic polymorphism around the arsenic methyltransferase (AS3MT, previously CYT19) gene, which has been supported by both genome-wide association studies (GWAS) and candidate gene studies.[48, 49] Environmental factors may also influence the arsenic metabolism though the underlying mechanisms remain unclear.[6, 50] Several external environmental factors including arsenic exposure dose, smoking, alcohol drinking, and nutrition status have been related to the individual variation of arsenic metabolism profile.[6, 50] Although there was no conclusive evidence to support causal relationships between these external factors and methylation capacity, recent progress has been made regarding the role of nutritional modification (e.g. folate supplementation) in enhancing arsenic methylation efficiency to mitigate arsenic toxicities.[51] On the other hand, internal environmental factors including age, sex, pregnancy, co-morbidities, and body mass index have been linked to the modification

of arsenic methylation capacity.[50, 52, 53] However, inconsistent results were reported in the current literature suggesting a need to refine study design and statistical methodology to better clarify the associations among arsenic metabolism, age, sex, pregnancy, and body fat composition.[50]

Health effects of arsenic metabolism

Certain patterns of arsenic metabolism have been linked to the risk of developing cancer, cardiovascular disease, and adiposity. For instance, higher MMA% and lower DMA% in urine has been related to increased risk of cancer[54-56] and cardiovascular disease.[57, 58] The increased risk of cancer and cardiovascular disease associated with higher MMA% in urine may be related to the high toxicity of MMA[III], which is also associated with insulin resistance in adipocytes.[59-63] DMA has long been regarded as a less toxic arsenic specie, although DMA [III] has been recently linked to the prevalence of diabetes in studies from Mexico and Bangladesh.[5, 64] However, recent research has also connected lower MMA% and higher DMA% with an increase in adiposity. As an up-to-date review of existing studies is lacking, we conducted a systematic review (Chapter 2) to describe key gaps in current arsenic metabolism research and inform future research needs and opportunities.

Crosstalk between arsenic metabolism and one-carbon metabolism.

The main biochemical pathway to facilitate the arsenic metabolism is one-carbon metabolism composed of three major units: folate cycle, methionine cycle, and transsulfuration pathway (figure 3).[65, 66] Perturbation of these cycles has been linked to increased risk of mortality, cancer, and cardiovascular diseases and may also influence the efficiency of arsenic metabolism.[65, 67-69] For malnourished populations in Bangladesh, the methylation capacity can be enhanced with short-term folate supplementation.[51] Conversely, arsenic exposure may also disturb the dynamic balance of one-carbon metabolism and its associated biochemical reactions including DNA methylation, redox regulation including homocysteine and glutathione metabolism, and other xenobiotic metabolic pathways.[6, 65, 70-72] To advance our understanding of how arsenic metabolism interacts with one-carbon metabolism, the fundamental step is to examine the association between arsenic metabolism and a spectrum of disease phenotypes related to one-carbon metabolism. This dissertation aims to inform biological meaning of arsenic metabolism and explore the complexity and challenges of the integrated risk assessment of arsenic.

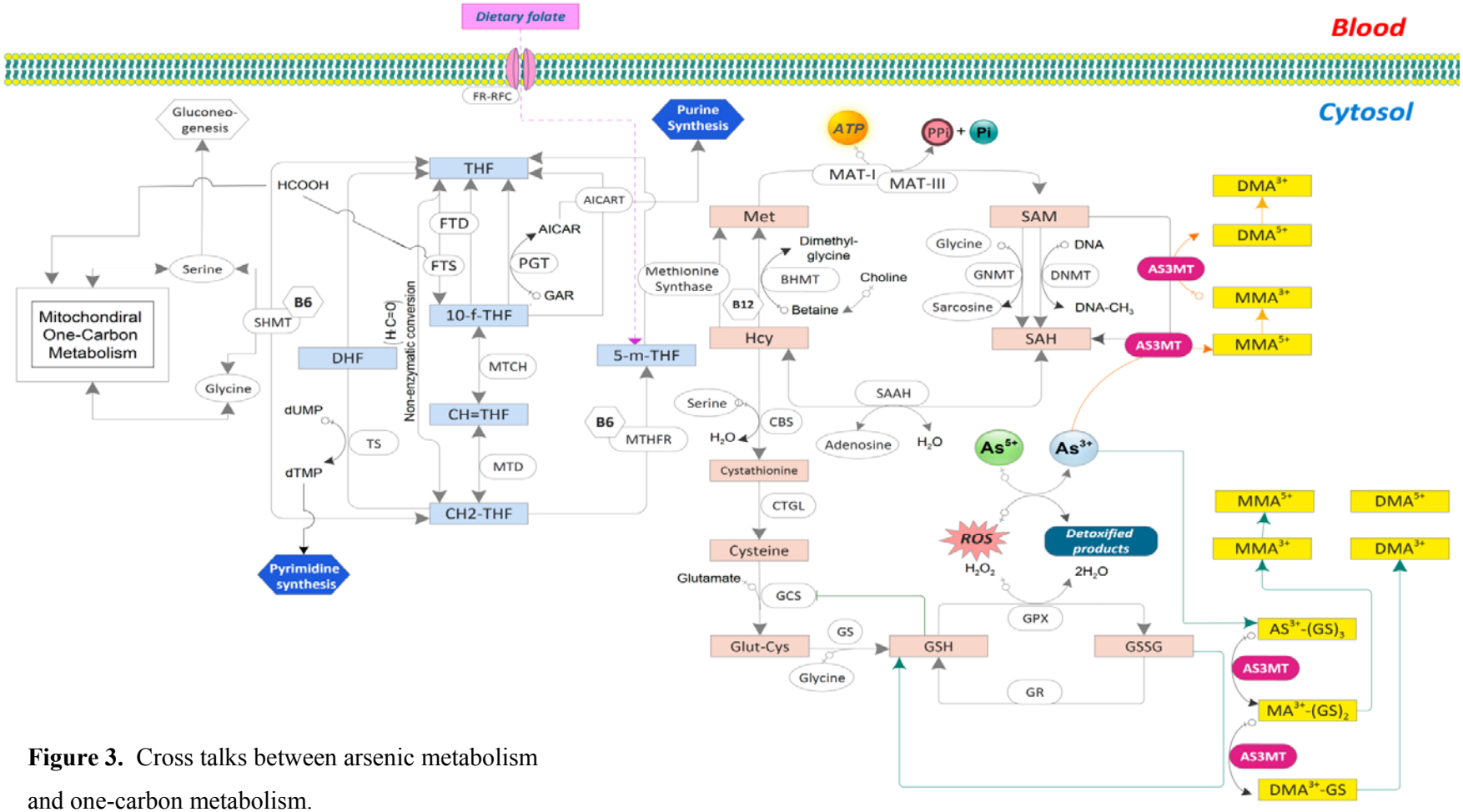


Figure 3. Cross talks between arsenic metabolism and one-carbon metabolism.

CHAPTER 1. Arsenic metabolism and chronic diseases: A systematic review and a metabolism variance analysis

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Abstract

Objective To systematically investigate the role of arsenic metabolism in the development of cancer, cardiovascular diseases, adiposity, and diabetic phenotypes and characterize the variation of arsenic metabolism in different populations worldwide.

Design Systematic review of observational studies

Data sources Medline/PubMed and EMBASE for relevant studies from inception to April 2014.

Eligibility criteria for selecting studies Observational studies that assessed the association between arsenic metabolism and health outcomes of interest including cancer, cardiovascular disease, and adiposity/diabetic phenotypes.

Results Twenty eight studies met the inclusion criteria, 12 on cancer, 9 on cardiovascular diseases, and 7 on adiposity and diabetic phenotypes. The median with interquartile range for iAs%, MMA%, and DMA% was 11.2 (7.8-14.9), 13(10.4-13.6), and 74.9(69.8-80.0), respectively. MMA% has the lowest inter-population variance and per doubling change in urine arsenic concentration was associated with 0.02% (95% CI, -0.7~0.6). For cancer, the patterns of a higher MMA% and a lower DMA% was associated with higher risk of developing all-site, urothelial, lung and skin cancers. For cardiovascular disease, a higher MMA% was associated with higher risk of carotid atherosclerosis and cardiovascular diseases but not hypertension. For adiposity and diabetic phenotypes, the pattern of a lower MMA% and a higher DMA% was associated with higher body mass index and higher metabolic syndrome risk.

Conclusion Although certain specific methylation patterns were identified to associate with disease risk, scopes and conclusions are constrained due to small sample size, limited prospective evidence, and inconsistent statistical approach. Relative constant MMA% across diverse populations is a novel finding from the mechanistic and evolutionary perspectives. More population evidence is needed to confirm our findings.

Introduction

Inorganic arsenic exposure through drinking water and food is a global environmental health problem.[1] Chronic arsenic exposure affects multiple organ systems resulting in various cancers and cardiovascular diseases, and maybe also in respiratory disease, diabetes, and kidney disease[2]. The World Health Organization (WHO) and U.S. Agency for Toxic Substances and Disease Registry (ATSDR) have ranked arsenic as top priority for rigorous risk assessment and exposure control.[3,4] Arsenic risk assessment, furthermore, is complicated by inter-individual variation in arsenic metabolism. After absorption, inorganic arsenic (arsenate and arsenite) is methylated into monomethylated and dimethylated arsenic compounds (MMA, DMA), which are then excreted through the kidney together with inorganic arsenic[5]. The average distribution of arsenic metabolites in urine has been reported to be 10-30% inorganic arsenic, 10-20% MMA and 60-80% DMA, with substantial inter-population and intra-population variations[6-9]. Higher levels of MMA% and lower levels of DMA% have been related to cancer and cardiovascular outcomes in populations from Taiwan, Bangladesh and Argentina[10-13]. Lower levels of MMA% and higher levels of DMA%, on the other hand, have been related to higher body mass index and metabolic syndrome[14-16].

Arsenic metabolism is tightly connected to one-carbon metabolism,[17] which is composed of three key cycles including the methionine cycle, the folate cycle, and the cysteine-cystathionine cycle[18,19]. The major methyl donor in the body, S-adenosylmethionine (SAM), is generated through the methionine cycle, to facilitate more than 50 methylation reactions in the body, including DNA methylation and arsenic methylation.[20,21] The methionine cycle is completed by the re-methylation of

homocysteine back to methionine, through the folate cycle,[22] or by irreversibly degrading homocysteine into cysteine.[23] Dysfunction of the methionine cycle has been linked to chronic diseases including cancer and cardiovascular disease.[19,24] The cysteine-cystathionine cycle involves the glutathione-transsulfuration pathway and generates anti-oxidant thio buffers, which are critical to maintain intracellular reduction-oxidation status.[25] This pathway may also assist arsenic methyltransferase (AS3MT) to reductively methylated inorganic arsenic[21]. The imbalance of redox status has also been linked to cancer metabolism[26,27], cardiovascular disease[28], and diabetes[29]. The intertwined relationship between arsenic metabolism and one-carbon metabolism may explain the individual susceptibility toward arsenic toxicity. Arsenic methylation pattern may also be used to estimate the gene-environment interaction.

An increasing number of studies have evaluated the role of arsenic metabolism in the development of cancer,[30,31] cardiovascular diseases,[13] adiposity[16], and diabetes[32]. The available evidence, however, has not been formally and comprehensively evaluated. Our study objectives were, first, to conduct a systematic review to examine the role of arsenic metabolism in the development of cancer, cardiovascular disease, diabetes and adiposity, and second, to characterize the arsenic metabolism in different populations worldwide.

Methods

Search strategy and study selection

The systematic search and review processes were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement criteria[33]. We searched PubMed/Medline and EMBASE for original

epidemiologic studies investigating the role of arsenic metabolism in the risk of cancer, cardiovascular disease, and diabetes. For arsenic metabolism, we used the following MeSH terms: “arsenic”, “methylation”, “metabolism”, “arsenic metabolism”, “arsenic methylation” combining with other specific text-word terms related to the key research concepts. During the screening, evidence investigating the association between arsenic metabolism and diabetes was limited. Therefore, we also included studies using adiposity or prediabetes as main end point of interest. (Supplementary table 1) shows the full search strategies. The search period was January 1966 through February 2014. There were no language restrictions. We also manually reviewed the reference lists from relevant original research.

Our primary exclusion criteria to screen records were: 1) publications contain no original research (reviews, editorials, non-research letters); 2) case reports and case series; 3) studies did not measure cancer, cardiovascular disease, diabetes (including prediabetes and metabolic syndrome), or adiposity (including obesity and body mass index); 4) studies did not have information on arsenic metabolism, as measured in urine (percentage [%] or ratios of urine arsenic metabolites). The secondary exclusion criteria included: 1) Lack of report of the association between arsenic metabolism and the study outcome; 2) Studies focused only on arsenic-related skin lesions (pre-malignancy); 3) pregnant population; 4) Duplicate study source population with same study design and outcome of interest.

To characterize the arsenic metabolism across human populations worldwide, we selected 19 studies from the current and the previous review[9] based on the following

criteria: 1) sample size larger than 30(2 studies were excluded); 2) For with studies with the same or overlapping population, we selected studies with largest sample size.

Data abstraction

Two authors, C.C. Kuo and C.W. Tsai, independently abstracted data from the articles that met the selection criteria. They developed a data extraction form to record the study characteristics (authors, journal, years of publication, country, study design, and objectives); the participant characteristics (study population (general vs. occupational) and number of participants); outcome definitions; measure of arsenic metabolism (% or ratio of urine arsenic metabolites, continuous and/or categorical); and the results of the association analysis from the statistical models adjusted for the most covariates. To assess study quality, we adapted the criteria used by Longnecker et al. for observational studies (supplementary figure 2-4). Two authors, C.C. Kuo and C.W. Tsai, also conducted risk of bias assessment for each study. The disagreement was resolved by consensus. The domains related to exposure assessment, outcome definition and statistical modeling including covariate adjustment were considered to be important for this review.

Statistical Methods

Study characteristics, population characteristics, outcome definitions, exposure measures, association measurements, and statistical models were summarized in a consistent manner. In each study, the following items were abstracted (or derived if not directly reported): the mean age and percentage of men, arsenic metabolism (percentage or ratio of urine arsenic metabolites) and urine total arsenic levels. For studies providing the median or geometric mean, the arsenic metabolism of the largest subgroup were used;

otherwise, data from pooled analyses were recorded. For studies only providing risk estimates among subgroups (interaction table), the relative risks were recalculated using pooled data. Both arithmetic and compositional geometric mean and variance were estimated. The relationship between total urine arsenic level and arsenic metabolism were evaluated using compositional data analysis (CoDa).[34] All statistical analysis were performed using packages, ggtern and compositions, in R 3.0.0.

Results

Twenty eight studies met the inclusion criteria, 12 on cancer, 9 on cardiovascular diseases, and 7 on adiposity and diabetic phenotypes (Supplementary figure 1). All studies were in English. The medians with interquartile range for iAs%, MMA%, DMA%, and total urine arsenic concentration were 11.2 (7.8-14.9), 13(10.4-13.6), 74.9(69.8-80.0), and 100(50.8-206.5), respectively. The compositional geometric mean for iAs%, MMA%, and DMA% was 11.3, 12.3, and 76.4, respectively. The distribution and variability of arsenic metabolism worldwide were summarized in figure 1 and 2. Individually, MMA% has the lowest inter-population variance (Figure 2). Compositionally, the largest variance occurred between iAs% and methylated arsenic species (MMA% and DMA%)(Supplementary figure 5). In univariable regression analysis, per doubling change in urine total arsenic level was associated with a 2.1% (95% CI, 0.8-3.5) increase in iAs%, 0.02% decrease (95% CI, -0.7~0.6) in MMA%, and a 1.9% (95% CI -3.7~-0.2) decrease in DMA%. The findings were consistent with the statistical results from compositional data analysis (Supplementary figure 6).

Cancer

Of the 12 studies, 3 were prospective cohort studies[10,35,36] and 9 were case-control studies. Only five studies were conducted outside of Taiwan (Table 1).[11,30,31,37,38] Of these five studies, the study populations were from Argentina and the United States[11,30], Chile[31], East Europe[38], and United States.[11,37] Studies from Putai township of Chiayi County and Tainan County in Taiwan (n=6) were from previous Blackfoot Disease (BFD) endemic area, characterized by high arsenic exposure in drinking water (>100 μg/L).[10,35,36,39-41] In contrast, only one study from Taipei City/County (n=1) was considered as low arsenic exposure (<100 μg/L in drinking water).[42] Study populations from Argentina, California and Nevada in the US, and Chile were exposed to low-to-moderate arsenic levels (<100 μg/L in drinking water).[11,31] Study populations from New Hampshire, US and Eastern Europe were exposed to low-to moderate arsenic levels (<100 μg/L).[37,38] A total of 5 studies evaluated the risk of urothelial cancers, five studies assessed skin cancer as the primary study outcome and two studies examined the risk of lung cancer(Table 1). One study from Taiwan reported the association between arsenic metabolism with all cancer incidence.[36,43] Most studies validated outcomes based on pathological and medical information (Table 1).

Arsenic metabolism was assessed based on the proportions of arsenic species in the urine (iAs%, MMA%, and DMA%) in 11 studies and based on the primary methylation index (PMI, the ratio of MMA over iAs) and secondary methylation index (SMI, the ratio of DMA over MMA) in 6 studies (all of them from Taiwan). One of three studies that reported PMI showed a positive association between PMI and the risk of urothelial carcinoma.[42] All three studies that reported SMI suggested a negative

association between SMI and urothelial cancers.[41,42,44] For iAs%, most studies from Taiwan showed a positive association between iAs% and cancer although one study conducted in Chile did not find the same pattern.[31] For MMA% and DMA%, five of 7 studies reporting both MMA% and DMA% supported that the pattern of a higher MMA% and a lower DMA% was associated with higher risk of developing all-site[36], urothelial[35,42], lung[31] and skin cancers.[38,40]

Cardiovascular disease

Five of nine enrolled studies were conducted in Taiwan[12,45-48], two from Bangladesh[13,49], and two from China(Table 2).[50,51]All study populations were considered to have high arsenic exposure(>100 μg/L in drinking water). Three of the nine studies were prospective cohort studies[13,47,48], and the other six were cross-sectional. The study outcomes included hypertension (n=4),[45,47,50,51] carotid atherosclerosis (n=3),[46,48,49] incident cardiovascular diseases (n=1)[13], and peripheral arterial disease (n=1).[12] Hypertension was defined using well-established guidelines. The diagnosis of carotid atherosclerosis and peripheral vascular disease were based on extracranial carotid Doppler ultrasound evaluation and ankle-brachial index (ABI), respectively.

Most studies from Taiwan and Bangladesh reported both iAs%, MMA%, and DMA% and methylation indices including PMI and SMI. For iAs% and DMA%, all studies showed no significant association with the cardiovascular outcomes of interest. For MMA%, there was no association with hypertension in all studies[45,47,50,51] ; however, a higher MMA% was associated with higher risk of cardiovascular diseases in Bangladesh.[13] Only one of 3 studies measuring intimal thickness supported that a higher MMA% was associated with higher risk of carotid atherosclerosis (Table 2).[49] Among 4

studies evaluating the association between methylation indices (PMI and SMI) and various cardiovascular outcomes, only one found a positive association of PMI and a negative association of SMI with incident CVD.[13]

Adiposity and diabetic phenotypes

Seven studies evaluated the associations between arsenic metabolism and adiposity and diabetes related outcomes (Table 3). All studies were published after 2010 and were in English. Study populations were from Bangladesh[32], Mexico[14,52,53], Taiwan[15,54], and the United States.[16] Two studies were case-control studies[15,32] and the rest of the studies were cross-sectional in design. Most study populations were exposed to moderate-high arsenic levels (>100μg/L in drinking water) except populations from Taipei, Taiwan[54] and the Strong Heart Study cohort (Arizona, Oklahoma, North/South Dakota, US).[16] Three studies evaluated diabetes or metabolic syndrome as the primary outcome[15,32,52], three studies assessed the association between arsenic metabolism and body mass index (BMI)[14,16,53], and one study reported the risk of having obesity in adolescents.[54] The definitions of diabetes were slightly different. The study from Mexico defined diabetes based on fasting glucose levels and self-reported physician diagnosis, while the study from Bangladesh used self-reported physician diagnosis exclusively.

In the two studies examining the relationship among diabetes, obesity and arsenic metabolism measured by proportions of arsenic metabolites in urine, there was no significant association.[32,54] However, a significant association between the pattern of a lower MMA% and a higher DMA% and higher metabolic syndrome risk was found in a study from Taiwan.[15] Three studies using BMI as primary end point all reported that lower MMA% associated with a higher BMI.[14,16,53]

Four studies reported PMI and SMI in addition to the proportions of arsenic species. Three of them evaluated the association between PMI and SMI with diabetes and the metabolic syndrome. Only one study found a lower PMI and a higher SMI were associated with the risk of metabolic syndrome.[15] A fourth study evaluated SMI with BMI, and found that higher SMI was associated with higher BMI.[14]

Discussion

This systematic review identified a significant gap between toxicological understanding of arsenic metabolism and its epidemiological application in risk assessment. Most studies had a small sample size and were assessed as being unclear or high risk of bias especially for studies were conducted based on exposure-by-subgroup interaction analysis. In addition, the lack of consensus over an appropriate standardized statistical model for estimating the arsenic metabolism makes the interpretation of the results challenging. Although most studies consistently supported a higher MMA% and a lower DMA% were associated with the risk of developing cancer, the result should be cautiously generalized to other populations as 7 of 12 enrolled studies were conducted in Taiwan from the same research group with significant overlaps in study population. For cardiovascular diseases, the research group in Bangladesh found a positive prospective association between MMA% and incident cardiovascular diseases and a positive cross-sectional association between MMA% and carotid atherosclerosis. However, no studies found any association between hypertension and arsenic metabolism. For adiposity, a consistent negative association between MMA% and BMI were found in all studies used BMI as the primary outcome measurement. However, the role of arsenic metabolism in

the development of diabetes and obesity remains inconclusive due to insufficient data and limited statistical power.

Substantial inter-population variability of arsenic metabolism has been recognized in two previous studies.[6,55] However, those studies analyzed few populations from China, Chile, Mexico, and Taiwan. In the current review, we found MMA% has less variability compared to iAs% and DMA% across diverse populations. In addition, MMA% is the only composition of arsenic metabolism that is not affected by the arsenic exposure (Supplementary figure 6). This finding implied that the first methylation process of arsenic in human body is tightly regulated and the second methylation process might play an adaptive role in the arsenic metabolism. Genetic polymorphism has been considered a major determinant in large inter-individual variability of the arsenic methylation pattern. The external factors such as arsenic exposure status may be equally important in adjusting arsenic metabolism and high arsenic exposure may impede the overall methylation capacity in human while also keep relative constant MMA%. More research is needed to explore the biological meaning of this unique phenomenon.

Several important observations remain to be explained. First, the interaction between arsenic exposure levels and arsenic metabolism is not clearly determined. For example, in studies of urothelial carcinoma or bladder cancer, a higher MMA% was associated with the risk of developing urothelial cancers even in the study populations with low levels of arsenic exposure (Taipei, Taiwan).[35,42] However, this pattern was only observed in studies of skin cancer with high arsenic exposure but not in a study with low arsenic exposure.[37] Second, the discrepancy in results between prospective studies and the cross-sectional studies in cancer and carotid atherosclerosis risk raise the concern that

disease outcome may modify the profile of arsenic metabolism. Future studies with large sample size, appropriate baseline arsenic metabolism estimation, and sufficient long-term follow-up may help verify the prospective association between arsenic metabolism and disease of interest. Third, interpretation of methylation indices (both PMI and SMI) is challenging. It is difficult to predict whether the numerator or the denominator will have dominant effect to drive the methylation indices. Developing a simple and interpretable modeling of arsenic metabolism is a research priority. Fourth, appropriate statistical modeling of arsenic metabolism remains unsettled. For instance, thirteen of 28 studies (46.4%) in our review adjusted arsenic exposure to evaluate the effect of arsenic metabolism. As arsenic exposure may be a potential confounder and also a strong risk factor for many outcomes of interest, adjusting arsenic exposure facilitates the interpretation of the statistical results. In addition, each composition of arsenic metabolism is constrained to 1 because they are normalized artificially to the sum of inorganic and methylated arsenic species. Handling compositional data using modern statistical methods designed for unconstrained data may lead to inappropriate inferences. Although compositional data analysis (CoDa) is almost unknown in the field of biomedical research and its applicability in biomedicine remains unclear, incorporating CoDa in the sensitivity analyses in arsenic metabolism research may be useful to gauge the robustness of the statistical results using conventional approaches.

The biological meaning of arsenic metabolism may be beyond the susceptibility of arsenic toxicity and may be the maker to estimate both genetic control of sensitivity to the environment and environmental control of gene expression (the gene-environment interaction). The interplay between one-carbon metabolism, arsenic metabolism, and

DNA methylation provides an opportunity to explore the genomic coding, metabolism regulation, and phenotype expression from a mechanistic perspective.[24] Increasing evidence supports the association between arsenic exposure and global DNA methylation status.[56,57] Moreover, mathematical modeling to handle the complexity of one-carbon metabolism and the interaction between the one-carbon and arsenic metabolism has been initiated.[58,59] The challenge of future research is to integrate metabolism modeling and epigenomics to evaluate current biomarkers and identify novel markers.

Conclusion

This is the first systematic review evaluating the current evidence examining the association between arsenic metabolism and different chronic disease outcomes. Although certain specific methylation patterns were identified as associated with increase disease risk, scopes and conclusions are constrained due to small sample size, limited prospective evidence, and inconsistent statistical approaches. Conducting large prospective cohort studies in populations exposed to a wide range of arsenic exposure levels is critical to better characterize the dynamic of arsenic metabolism and factors that influence the individual metabolism patterns. Relative constant MMA% across diverse populations is a novel and interesting finding from the mechanistic and evolutionary perspectives. More population evidence is needed to confirm this finding. Using a family-based case-control study is important to investigate the role of candidate-gene in arsenic toxicity and arsenic metabolism as such a study design would effective eliminate the concern of population stratification confounding.[60] Understanding the biological and epidemiological meaning of arsenic metabolism could significantly improve the risk

assessment of arsenic toxicity and provide a potential tool for disease prediction, prevention and control.

Table 1. Studies of arsenic metabolism and cancer.

1st author, year	Design Cases/ Non Case	Population - Men (%) - Age range	Outcome	Arsenic exposure - Sample - Level	iAs% - Mean(SD) - Highest vs. lowest categories -RRe (95% CI)	MMA% - Mean (SD) - Highest vs. lowest categories - RRe (95% CI)	DMA% - Mean (SD) - Highest vs. lowest categories - RRe (95% CI)	PMI - Mean (SD) - Highest vs. lowest categories - RRe (95% CI)	SMI - Mean (SD) - Highest vs. lowest categories - RRe (95% CI)	Adjustment Factors
Chung 2009	CO 17/191	Putai, Taiwan - 36.1% - Mean 46.5 yrs	All-site cancer (cancer registry)	- Urine - mean total arsenic 78.2 µg/L	- 7.5 (7.5) - ≥5.9 vs. <5.9 - 0.6 (0.2-1.7)	- 13.0 (8.9) - . ≥10.8vs. <10.8 - 2.4 (0.8-9.1)	- 79.5 (12.7) - ≥ 82.4 vs <82.4 - 0.9 (0.3-2.7)	NR	- 9.4 (8.4) - ≥ 7.4 vs. <7.4 - 0.4 (0.1-1.4)	Age, sex, education
Chen 2003	CC 49/224	Tainan, Taiwan - 63.0% - ≥30 yrs	Urothelial carcinoma (pathology)	- Water - mean CAE 8.5 (mg/L-year)	NR	NR	NR	- 4.8 - >0.9 vs. ≤0.9 - 0.7 (0.4-1.5)	- 10.9 - >4.8 vs. ≤4.8 - 0.6 (0.3-1.2)	Age, sex, education, smoking, body mass index, hair dye, CAE
Steinmaus 2006	CC 114/114	Córdoba, Argentina - 82.5% - 20-80 yrs	Urothelial carcinoma (pathology)	- Water - low-moderate	- 16.1 (10.0) - NR	- 14.6 (9.7) - ≥16.7 vs. <16.7 - 1.33 (0.74-2.39)	- 69.3 (16.3) - NR	NR	NR	Age, sex, smoking, bombilla use
	23/49	California and Nevada, US - 80.6% - 40-85 yrs	Urothelial carcinoma (pathology and cancer registry)	- Water - low-moderate	- 11.9 (4.9) - NR	- 13.2 (4.1) - ≥14.9 vs. <14.9 - 1.19 (0.38-3.68)	- 74.9 (6.9) - NR			Age, sex, smoking

Pu 2007	CC 177/313	Taipei, Taiwan - 66.3% - 24-93 yrs	Urothelial carcinoma (pathology)	- Urine - mean sum iAs, MMA, and DMA 30.0 (µg/g creatinine)	- 5.9 (6.9) - >5.2 vs. ≤2.4 - 1.2 (0.7-2.0) - *p*-trend=0.7	- 9.9 (10.1) - >9.2 vs. ≤3.0 - 2.8 (1.6-4.8) - *p*-trend <0.0001	- 84.2 (12.6) - >92.5 vs. ≤85.0 - 0.4 (0.2-0.7) - *p*-trend =0.004	- 3.3 (11.3) - >2.0 vs. ≤0.3 - 3.1 (1.7-5.6) - *p*-trend <0.001	- 12.9 (29.7) - >12.7 vs. ≤4.8 - 0.3 (0.2-0.6) - *p*-trend=0.001	Age, sex, education, alcohol, ethnicity, pesticide usage
Huang 2008	CO 37/928	Putai, Taiwan - 43% - >30 yrs	Urothelial carcinoma (cancer registry)	- Urine - Median sum iAs, MMA, and DMA 63.7 µg/L	- 5.9 - ≥8.0 vs. <4.3 - 1.4 (0.5-3.6) - *p*-trend=NS	- 11.3 - ≥15.6 vs. <8.4 - 1.7 (0.7-4.0) - *p*-trend=NS	- 81.4 - ≥85.7 vs. <76.0 - 0.3 (0.1-0.9) - *p*-trend=<0.05	- 1.9 - ≥ 2.8 vs. <1.3 - 0.8 (0.4-2.0) - *p*-trend=NS	- 7.0 - ≥9.8 vs. <4.8 - 0.5 (0.2-1.3) - *p*-trend=NS	Age, sex, education, smoking, CAE
Melek 2014	CC Lung ca 94; uro-thelial ca 117; non-case 347	Northern Chile - 69.0% - >25 yrs	Urothelial carcinoma (medical records) Lung cancer (medical records)	- Water - Lifetime average arsenic 109.3 µg/L	- 9.6 (6.3) - ≥10.8 vs. <10.8 - Urothelial ca. 0.3 (0.2-0.5) - Lung ca. 1.1 (0.7-1.8)	- 10.8 (4.8) - >12.5 vs. <8.5 - Urothelial ca. 2.0 (1.2-3.5) - Lung ca. 3.3 (1.8-6.0)	- 80.0 (8.3) - ≥83.9 vs. <83.9 - Urothelial ca. 1.7 (1.1-2.6) - Lung ca. 0.6 (0.4-1.1)	NR	NR	Age, sex, smoking
Steinmaus 2010	CC 45/75	Córdoba, Argentina - 88.3% - 20-80 yrs	Lung cancer (medical records)	- Water - low-moderate	- 15.7 (5.5) -NR	- 15.5 (6.7) - ≥17.2 vs. <11.8 - 2.6 (1.0-6.5) - *p*-trend=0.04	- 68.7 (9.6) - NR	NR	NR	Age, sex, smoking, drinking water arsenic exposure

Hsueh 1997	CO 33/621	Putai, Taiwan - 42% - >30 yrs	Skin cancer (pathology)	- Water - Artesian well 700-930 μg/L	- 10.7 (6.2) - NR	- 24.6 (11.2) - CAE>20 & MMA%>26.7 vs. CAE≤20 & MMA%≤26.7 - 24.0 (2.6-225.2)	- 64.7 (13.3) - NR	- 3.3 (2.3) - NR	- 3.6 (4.6) - NR	Age, sex, β-carotene
Yu 2000	CC 26/26	Putai, Taiwan - 26.9% - Mean 63.4 yrs	Skin cancer* (dermatologists' diagnosis)	- Urine - Mean sum iAs, MMA, and DMA 55.7 ppb	- 12.3 - >2.27 vs ≤2.27 - 3.5 (0.7-16.9)	- 15.5 - >15.5 vs ≤15.5 - 5.5 (1.2- 24.8)	- 72.2 - <72.2 vs ≥72.2ς - 3.25 (1.1-10.0)	NR	NR	Age, sex
Chen 2003	CC 76/224	Tainan, Taiwan - 59.7% - >30 yrs	Skin cancer (pathology)	- Urine - Mean sum iAs, MMA, and DMA 43.5 μg/L	- 7.4 (6.6) - NR	- 13.0 (10.9) - Highest vs. lowest tertile - 1.4 (0.6-3.4) - *p*-trend=NS	- 79.6 (16.3) - Highest vs. lowest tertile - 0.8 (0.3-1.9) - *p*-trend=NS	- 4.3 (8.8) - >3 vs.≤ 1 - 1.3 (0.6-2.9) - *p*-trend=NS	- 10.7 (11.5) - >9.4 vs. ≤ 5 - 0.9 (0.4-2.1) - *p*-trend=NS	Age, sex, education, smoking, alcohol, body mass index, sun exposure, CAE
Gilbert-Diamond 2013	CC 470/447	New Hampshire, US -59.1% - 25-74 yrs	Squamous cell carcinoma (pathology)	- Urine - Mean sum iAs, MMA, and DMA 5.0 μg/L	- ~6.4 - continuous variable - No association	- ~9.7 - continuous variable - No association	- ~80.8 - continuous variable - No association	- ~1.5 - continuous variable - No association	- ~8.3 - continuous variable - No association	Age, sex, education, smoking, body mass index, urine creatinine, skin reaction to sun exposure, water arsenic
Leonardi 2012	CC 529/540	Hungary, Romania, and Slovakia - 48.2 % - 30-79 yrs	Basal cell carcinoma (pathology)	- Water - Lifetime average iAs conc. 1.2 μg/L (median)	- 7.6 - NR	- 15.8 - Per 10μg/L lifetime iAs conc. Increase among MMA%<15.8 1.0 (0.9-1.2) ; MMA% ≥15.8 1.2 (1.1-1.4)	- 76.6 - Per 10μg/L lifetime iAs conc. Increase among DMA%<76.6 1.2 (1.1-1.4) ; DMA% ≥76.6 1.0 (0.9-1.2)	NR	NR	Age, sex, education, skin response to 1-hr midday sun, skin complexion, country

Note: *:Skin cancer refers to non-melanoma skin cancer; ς: Lowest vs. highest categories.

Abbreviations of Table 1: CAE, cumulative arsenic exposure; CC, case control study; CO, prospective cohort study; DMA, dimethylarsinate; iAs, inorganic arsenic; MMA, monomethylarsonate; PMI, primary methylation index(MMA/iAs); SMI, secondary methylation index (DMA/MMA); RRe, estimated relative risk; SD, standard deviation; CI, confidence interval; NR, not reported ; NS, not significant

Table 2. Studies of arsenic metabolism and cardiovascular diseases.

1st author, year	Design -Sample size (n)	Population - Men (%) - Age range	Outcome	Arsenic exposure - Sample - Level	iAs% - Mean(SD) - Highest vs. lowest categories -RRe (95% CI)	MMA% - Mean (SD) - Highest vs. lowest categories - RRe (95% CI)	DMA% - Mean (SD) - Highest vs. lowest categories - RRe (95% CI)	PMI - Mean (SD) - Highest vs. lowest categories - RRe (95% CI)	SMI - Mean (SD) - Highest vs. lowest categories - RRe (95% CI)	Adjustment Factors
Tseng 2005	CS 54/425	Putai, Taiwan - 45.9% - ≥30 yrs	Peripheral vascular disease (ankle-brachial indices)	- Urine - Mean sum iAs, MMA, and DMA 75.8 μg/L	- 7.9 (6.8) - NR	- 13.7 (8.2) - NR	- 78.4 (11.1) - NR	- 2.9 (7.9) -NR	- 9.9 (15.0) - NR	Age, sex, alcohol, body mass index, cholesterol
Wu 2006	NCC 163/163	Ilan, Taiwan - 54.3% - ≥40 yrs	Carotid atherosclerosis(IMT ≥1.0mm or the presence of ECCA plaque)	- Water - <0.15 ~ 3590 μg/L	NR	- NR - ≥ 16.5 vs. <9.9 - 1.1 (0.6-1.9)	NR	NR	NR	Age, sex, smoking, total cholesterol, hypertension, CAE
Huang 2007	CS 372/499	Putai, Taiwan - 44.0% - ≥30 yrs	Hypertension (a mean SBP ≥140 mmHg or a mean DBP ≥ 90 mmHg)	- Urine - Mean sum iAs, MMA, and DMA 76.6 μg/L	- 8.3 (7.6) - ≥ 8.0 vs. <4.5 -1.2 (0.8-1.9)	- 13.6 (8.7) - ≥15.5 vs. <8.1 - 1.0 (0.7-1.6)	-78.1 (12.2) - ≥85.3 vs. <75.8 - 1.1 (0.7-1.6)	- 3.1 (8.0) - ≥2.7 vs. <1.2 -0.9 (0.6-1.3)	- 11.7 (31.0) - ≥9.8 vs. <4.9 - 1.1 (0.7-1.7)	Age, sex, smoking, alcohol, body mass index, triglyceride, CAE
Huang 2009	CS 121/183	Putai, Taiwan - 52.0% - ≥30 yrs	Carotid atherosclerosis (IMT ≥1.0mm or the presence of ECCA plaque)	- Urine - Mean sum iAs, MMA, and DMA 79.6 μg/L	- 7.2 (7.4) - Case vs. control - No difference	- 14.0 (8.7) - Case vs. control - No difference	- 78.9 (11.9) - Case vs. control - No difference	- 3.3 (4.5) - Case vs. control - No difference	- 13.3 (47.1) - Case vs. control - No difference	Age, sex, smoking, hypertension, diabetes, total cholesterol, total urine arsenic

Wang 2011	CO 110/242	Putai, Taiwan - 42.1% - ≥40 yrs	Hypertension (a mean SBP ≥140 mmHg or a mean DBP ≥ 90 mmHg or on anti-HTN medication)	- Water - mean CAE 11.8 (mg/L-year)	- NR - >17 vs. <10 - 0.7 (0.3-1.6)	- NR - >12 vs. < 6 - 0.6 (0.3-1.3)	- NR - >0.81 vs. <0.71 - 1.4 (0.6-3.2)	NR	NR	Age, sex, body mass index, glucose
Chen 2013	CS 959	Araihazar, Bangladesh - 40.3% - ≥18 yrs	Carotid IMT as a continuous dependent variable	- Urine - Mean sum iAs, MMA, and DMA 259.5 µg/g cre	- 15.5 - Per 10% change - β=4.1 (-4.1-12.3)	- 13.0 - Per 10% change - β=12.1 (0.4-23.8)	- 71.6 - Per 10% change - β=-6.3 (-12.8-0.2)	- 0.98 - per 1-unit change - β=1.5 (-5.2-8.1)	-6.7 - per 1-unit change - β=-1.2 (-2.8-0.4)	Age, sex, education, smoking, body mass index, systolic blood pressure, diabetes
Chen 2013	CCO 369/1,109 (subcort)	Araihazar, Bangladesh - 50.6 % - ≥18 yrs	Fatal and nonfatal cardiovascular disease (medical records, death certificate, outcome assessment committee)	- Urine - Mean sum iAs, MMA, and DMA 272.3 µg/g creatinine	- 15.5 (6.9) - [17.4-69.3] vs. [0.3-12.4] - 1.1 (0.7-1.6)	- 13.2 (5.2) - [14.4-33.8] vs. [0.2-10.3] - 1.6 (1.1-2.2)	- 71.3 (8.7) - [75.6-99.2] vs. [27.9-68.6] - 0.8 (0.5-1.1)	- 1.0 (0.8) - [1.06-19.57] vs [0.01-0.66] - 0.9 (0.6-1.3)	- 6.0 (3.1) - [7.2-32.3] vs. [1.4-4.8] - 0.5 (0.3-0.9)	Age, sex, education, smoking, alcohol, specific gravity-corrected arsenic level
Li 2013	CS 182/487	Inner Mongolia, China - 42.6% - Mean 49.7 yrs	Hypertension (a mean SBP ≥140 mmHg or a mean DBP ≥ 90 mmHg or on anti-HTN medication)	- Water - geometric mean CAE 0.02-2.9 (mg/L-year)	- 6.3-11.4 - per % change - Water arsenic levels (µg/L) **<10:** 0.8 (0.3-2.3) **10-50:** 1.5 (0.6-3.9) **>50:** 2.0 (1.0-4.1)	- 12.5-14.0 - per % change - Water arsenic levels (µg/L) **<10:** 0.5 (0.05-4.5) **10-50:** 0.8 (0.1-7.1) **>50:** 1.8 (0.4-7.9)	- 67.5-73.9 - per % change - Water arsenic levels (µg/L) **<10:** 0.8 (0.1-6.9) **10-50:** 0.1 (0.002-4.4) **>50:** 0.04 (0.002-0.8)	NR	NR	Age, sex, smoking, alcohol, body mass index, diabetes

Li 2013	CS 168/436	Shanxi, China -42.2% -Mean 49.5	Hypertension (a mean SBP ≥140 mmHg or a mean DBP ≥ 90 mmHg or on anti-HTN medication)	- Urine - geometric mean sum iAs, MMA, and DMA 135.6-178.3 µg/g cre	- 9.5-9.6 - >15.8 vs. <7.3 - 1.5 (0.9-2.5)	- 12.8-13.3 - >16.4 vs. <11.9 - 1.00 (0.-1.7)	-69.5 - >79.1 vs. < 68.7 - 0.7 (0.4-1.2)	NR	NR	Age, sex, smoking, alcohol, body mass index

Abbreviations: CAE, cumulative arsenic exposure; CO, prospective cohort study; CCO, case-cohort study; CS, cross-sectional study; NCC, nested case control study; DMA, dimethylarsinate; iAs, inorganic arsenic; MMA, monomethylarsonate; PMI, primary methylation index(MMA/iAs); SMI, secondary methylation index (DMA/MMA); IMT, intimal-medial thickness; ECCA, extra-cranial carotid artery; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; RRe, estimated relative risk; SD, standard deviation; CI, confidence interval; NR, not reported

Table 3. Studies of arsenic metabolism, adiposity and diabetes

1st author, year	Design Cases/ NC	Population - Men (%) - Age range	Outcome	Arsenic exposure - Sample - Level	iAs% - Mean(SD) - Highest vs. lowest categories -RRe (95% CI)	MMA% - Mean(SD) - Highest vs. lowest categories -RRe (95% CI)	DMA% - Mean(SD) - Highest vs. lowest categories -RRe (95% CI)	PMI - Mean(SD) - Highest vs. lowest categories -RRe (95% CI)	SMI - Mean(SD) - Highest vs. lowest categories -RRe (95% CI)	Adjustment Factors
Gomez-Rubio 2011	CS 624	Arizona, USA and Sonora, Mexico - 0% - ≥6 yrs	MMA% and DMA/MMA (SMI)	- Urine - Mean sum iAs, MMA, and DMA 53.6 µg/L	- ~12.1 - NR	- ~10.6 - dependent variable - β of BMI =-0.02 (*p* value <0.01)	- ~77.4 - NR	NR	- ~8.5 - dependent variable - β of BMI =0.02 (*p* value <0.01)	Age, smoking, ethnicity, location, total urine arsenic level, AS3MT7388, AS3MT M287T
Del Razo 2011	CS 25/233	Zimapán and Lagunera, Mexico - 32.6% - ≥5 yrs	DM (FBG ≥126 mg/dL, OGTT ≥200 mg/dL, self-reported diagnosis, or DM medication)	- Urine - Mean sum iAs, MMA, and DMA 41.2 µg/L	NR	NR	NR	- 1.3 - per IQR change - 1.0 (0.9-1.1)	- 5.6 - per IQR change - 1.4 (0.9-2.1)	Age, sex, obesity, hypertension
Su 2012	CS 101/202	Taipei, Taiwan - 53.1% - 6-12 yrs	Obesity (National guideline)	- Urine - Mean sum iAs, MMA, and DMA 30.0 µg/g creatinine	- 4.4 - Obese vs. normal weight - No difference	- 4.8 - Obese vs. normal weight - No difference	- 90.5 - Obese vs. normal weight - No difference	NR	NR .	–
Gomez-Rubio 2012	CS 746	Sonora, Mexico - 32.3% - ≥6 yrs	MMA%	- Urine - Mean sum iAs, MMA, and DMA 170.4 ppb	- 14.3	- 11.6 (4.2) - dependent variable - Women (506) - β of BMI =-0.02 (*p* value <0.01)	- 74.1	NR	NR	Age, indigenous American proportion, haplotypes of alleles7388 and M287T, total urine arsenic

Chen 2012	CC 111/136	Putai, Taiwan - 42.9% - ≥40 yrs	Metabolic syndrome (meet 3 or more Adult Treatment Panel III criteria)	- Urine - Mean sum iAs, MMA, and DMA 43.5 µg/g creatinine	- 17.0 (16.2) - NR	- 10.1 (9.0) - >11.3 vs. <5.8 - 0.35 (0.2-0.7)	- 72.8 (19.1) - >81.9 vs. <72.4 - 2.0 (1.1-3.9)	- 0.8 (0.7) - >0.9 vs. <0.4 - 0.4 (0.2-0.8)	- 17.9 (31.9) - >12.0 vs. <6.1 - 2.6 (1.4-5.1)	Age, betal nut chewing
Nizam 2013	CC 140/180	Faridpur, Bangladesh - 40.6% - ≥20 yrs	DM (self-reported diagnosis and HbA1c ≥ 7%)	- Urine - Mean sum iAs, MMA, and DMA 242.6 µg/L	- 9.6 - DM vs. non-DM - No difference (*p* value =0.35)	- 9.4 - DM vs. non-DM - No difference (*p* value =0.08)	- 81.0 - DM vs. non-DM - No difference (*p* value =0.14)	- 1.3 - DM vs. non-DM - No difference (*p* value =0.37)	- 10.6 - DM vs. non-DM - No difference (*p* value =0.09)	Age, sex, smoking, body mass index, family history of diabetes, income, duration of drinking water, water arsenic
Gribble 2013	CS 3,663	Arizona, Oklahoma, N/S Dakota, USA (Strong Heart Study) - 41.1% - 45-74 yrs	iAs% MMA% DMA%	- Urine - Mean sum iAs, MMA, and DMA ~10 µg/L	- 7.9 - BMI ≥ 35 vs. <25 - Difference of mean iAs% - -1.7 (-2.2~-1.2)	- 13.9 - BMI ≥ 35 vs. <25 - Difference of mean MMA% - -4.0 (-4.5~-3.5)	- 77.8 - BMI ≥ 35 vs. <25 - Difference of mean DMa% - 5.7 (4.8~6.5)	NR	NR	Age, sex, education, smoking, alcohol, specific gravity-corrected urine arsenic

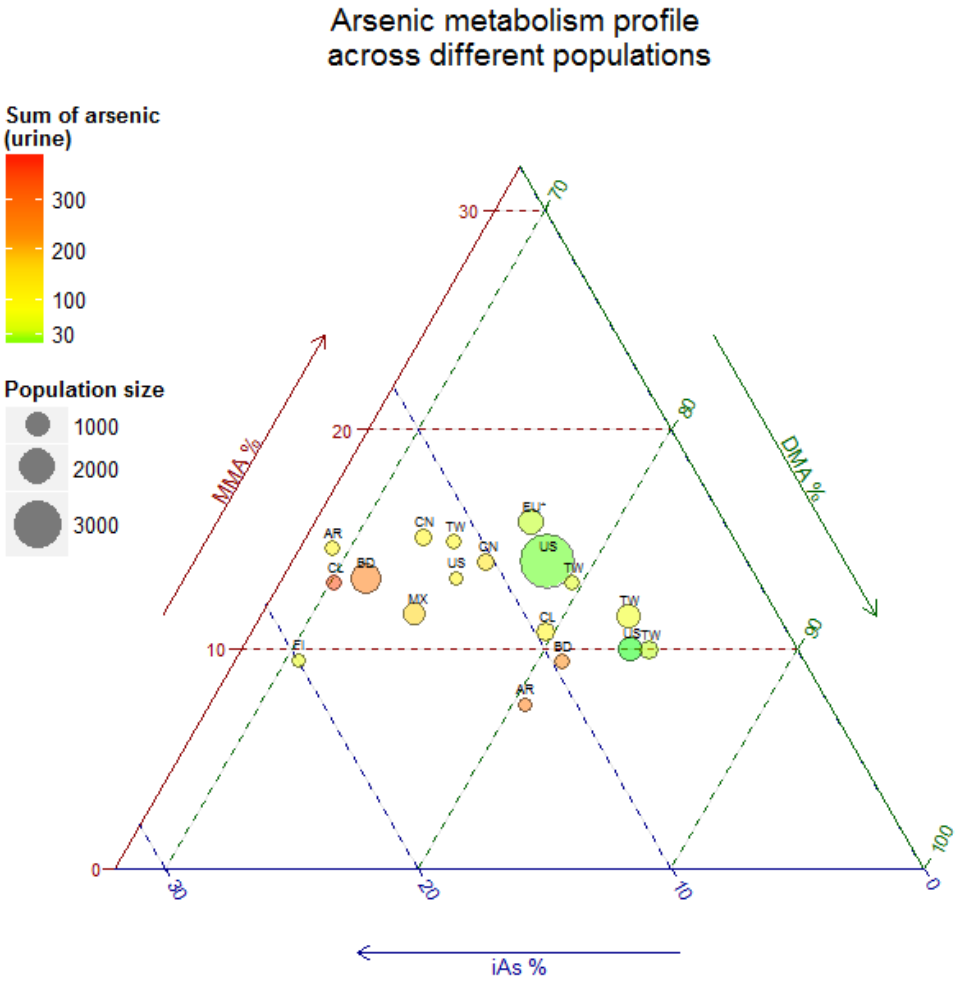
Abbreviations: BMI, Body mass index (kg/m^2); CC, case-control study; CS, cross-sectional study; DMA, dimethylarsinate; iAs, inorganic arsenic; MMA, monomethylarsonate; PMI, primary methylation index(MMA/iAs); SMI, secondary methylation index (DMA/MMA); DM, diabetes mellitus; FBG, fasting blood glucose; OGTT, oral glucose tolerance test; IMT, intimal-medial thickness; HbA1c, hemoglobin A1c; RRe, estimated relative risk; SD, standard deviation; CI, confidence interval; NR, not reported

Table 4. Summary of arsenic methylation pattern for different health outcomes.

Health outcomes	iAs%	MMA%	DMA%	PMI	SMI
Cancer					
All-site cancer					
Chung 2009	↓	↑	↓	NR	↓
Lung cancer					
Steinmaus 2010	NR	↑↑	NR	NR	NR
Melek 2014	↑	↑↑	↓	NR	NR
Skin cancer					
Hsueh 1997	NR	↑	NR	NR	NR
Yu 2000	↑	↑↑	↓↓	NR	↔
Chen 2003	NR	↑	↓	↑	↓
Leonardi 2012	NR	↑	↔	NR	NR
Gilbert-Diamond 2013	↔	↑	↓	↑	↔
Urothelial cancer					
Chen 2003	NR	NR	NR	↓	↓
Steinmaus 2006	NR	↑	NR	NR	NR
Pu 2007	↑	↑↑	↓↓	↑↑	↓↓
Huang 2008	↑	↑	↓	↓	↓
Melek 2014	↓	↑↑	↑↑	NR	NR
Cardiovascular disease					
Chen 2013	↑	↑↑	↓	↓	↓
Carotid atherosclerosis					
Wu 2006	NR	↑	NR	NR	NR
Huang 2009	↓	↑	↓	↑↑	↓
Chen 2013	↑	↑↑	↓	↑	↓
Hypertension					
Huang 2007	↑	↔	↑	↓	↑
Wang 2011	↓	↓	↑	NR	NR
Li 2013	↑	↑	↓	NR	NR
Li 2013	↑	↔	↑	NR	NR
Adiposity and diabetes					
Body mass index					
Gomez-Rubio 2011	NR	↓↓	NR	NR	↑↑
Gomez-Rubio 2012	NR	↓↓	NR	NR	NR
Gribble 2013	↓↓	↓↓	↑↑	NR	NR
Obesity					
Su 2012	↓	↓	↑	NR	NR
Metabolic syndrome					
Chen 2012	NR	↓↓	↑↑	↓↓	↑↑
Diabetes mellitus					
Del Razo 2011	NR	NR	NR	↔	↑
Nizam 2013	↓	↓	↑	↔	↑

Note: arrows stand for the direction of association; double arrows stand for the direction of significant association ($p<0.05$); ↔, null association; NR, not reported.

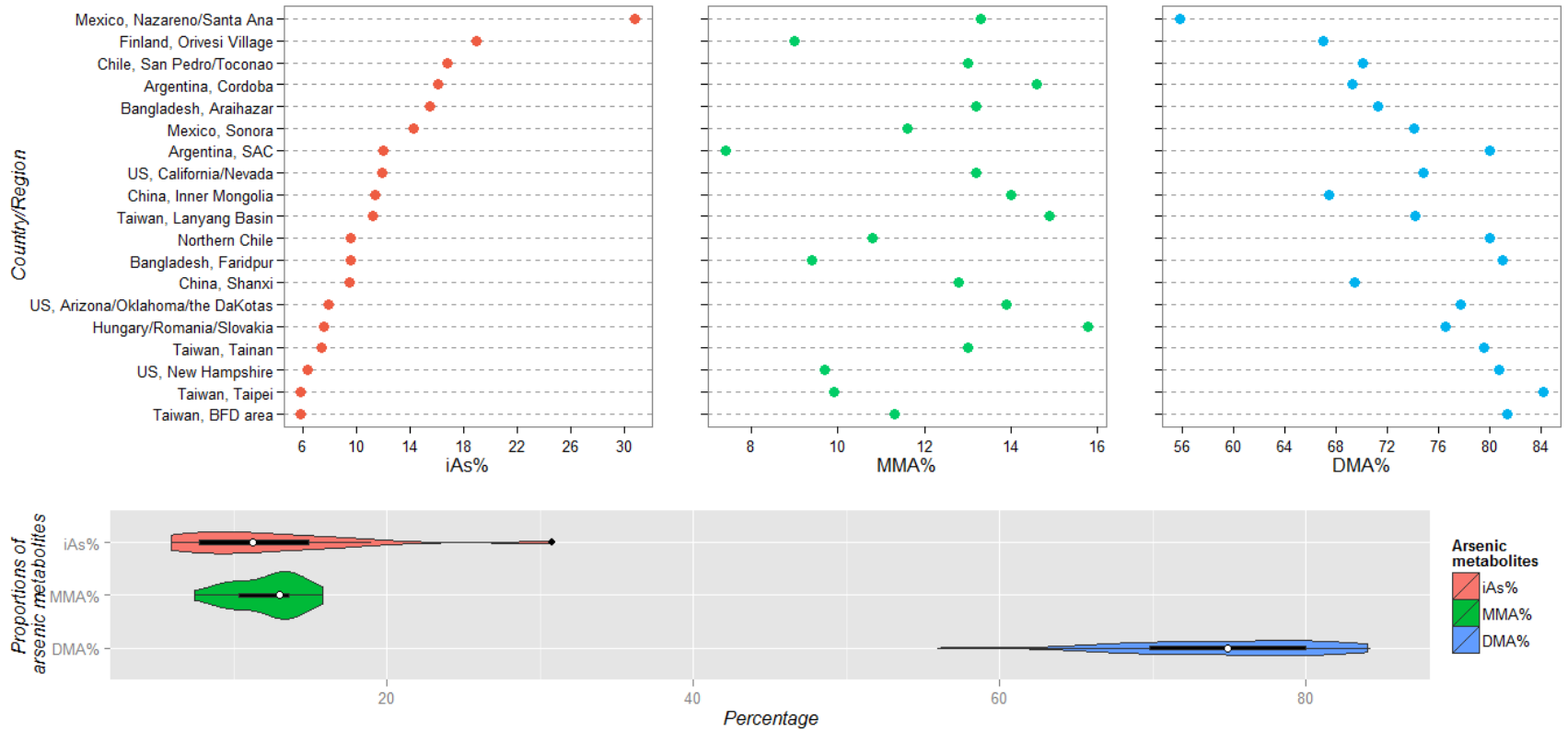
Figure 1. The distribution of arsenic metabolism across populations worldwide.



Population selection criteria:

1. Studies with sample size>30
2. For duplicate study population, the largest studies were selected.
3. For studies providing median or geometric mean, the arsenic metabolism profile of the largest subgroup was selected, otherwise, pooled data were used.
4. Six studies (not in our review) were incorporated according to a previous review.[9]

Figure 2. The variability of arsenic metabolism across populations worldwide.



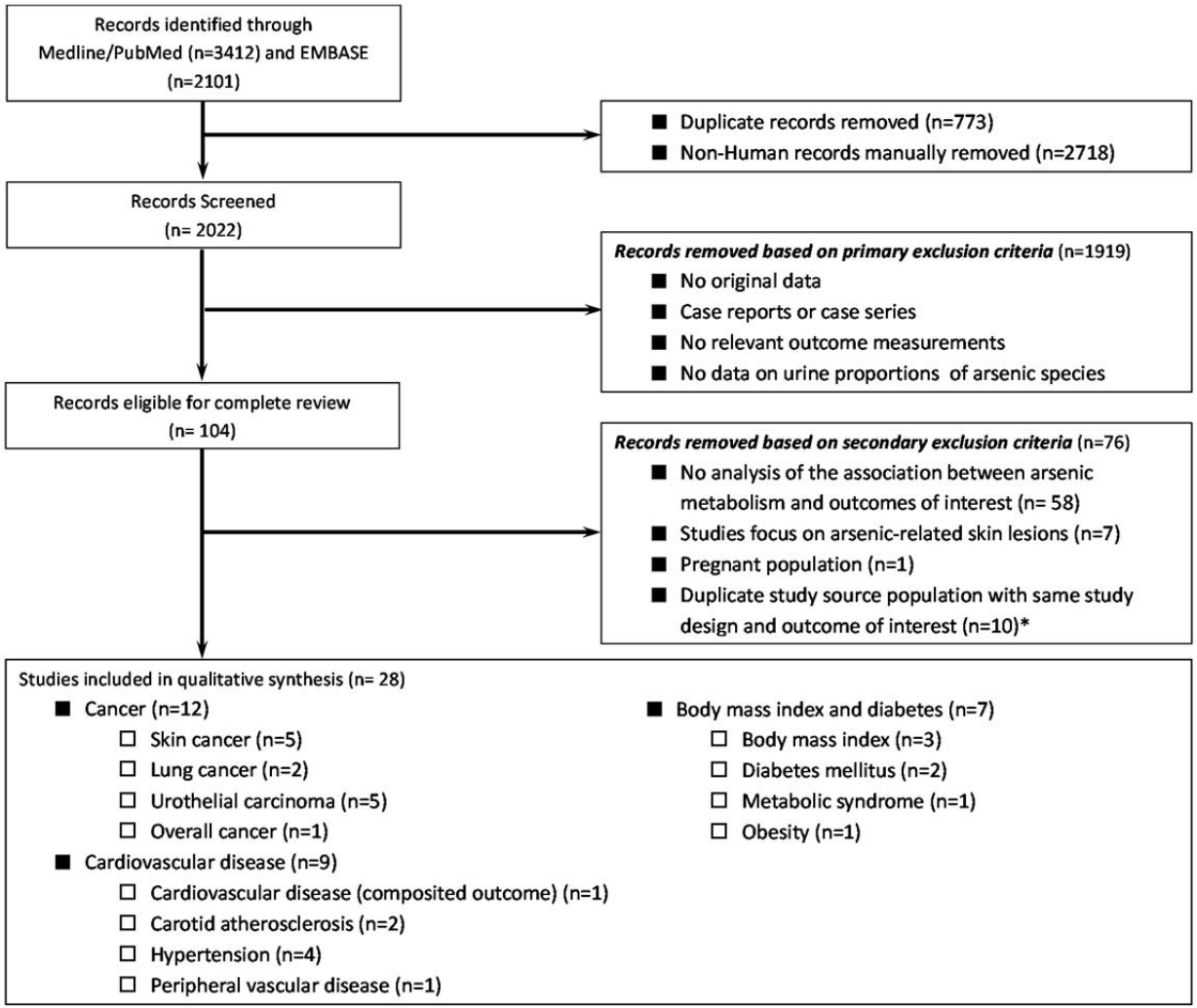
Supplementary table 1. PubMed and EMBASE database search strategies for arsenic metabolism and outcomes of interest

Database	Medline/PubMed
Date	April 3, 2014
Strategy	We combined the results for three major concepts(#1 to #3 below using "AND") with restriction to human studies (#4 using "NOT")
#1. Arsenic [28,732]	"Arsenic"[Mesh] OR "Arsenic Poisoning"[Mesh] OR "Arsenicals"[Mesh] OR "Arsenic"[tw] OR "Arsenic Poisoning"[tw] OR "Arsenicals"[tw] OR arsenite OR arsenate OR arsenicals
#2. Metabolism [6,265,772]	"Metabolism"[Mesh] OR "metabolism" [Subheading] OR "Metabolic Networks and Pathways"[Mesh] OR "Carbohydrate Metabolism"[Mesh] OR "Lipid Metabolism"[Mesh] OR "Glucose Metabolism Disorders"[Mesh] OR "Secondary Metabolism"[Mesh] OR "Lipid Metabolism Disorders"[Mesh] OR "Pyruvate Metabolism, Inborn Errors"[Mesh] OR "Purine-Pyrimidine Metabolism, Inborn Errors"[Mesh] OR "Phosphorus Metabolism Disorders"[Mesh] OR "Metal Metabolism, Inborn Errors"[Mesh] OR "Amino Acid Metabolism, Inborn Errors"[Mesh] OR "Steroid Metabolism, Inborn Errors"[Mesh] OR "Iron Metabolism Disorders"[Mesh] OR "Metabolism, Inborn Errors"[Mesh] OR "Lipid Metabolism, Inborn Errors"[Mesh] OR "Energy Metabolism"[Mesh] OR "Carbohydrate Metabolism, Inborn Errors"[Mesh] OR "Calcium Metabolism Disorders"[Mesh] OR "Methylation"[Mesh] OR "DNA Methylation"[Mesh] OR "Arsenic metabolism" OR "Arsenic methylation"
#3. Health outcome measures [7,339,336]	**[Cancer]** cancer[sb] OR **[Cardiovascular diseases]** "Atherosclerosis"[Mesh] OR "Carotid Artery Diseases"[Mesh] OR "Coronary Artery Disease"[Mesh] OR "Cardiovascular Diseases"[Mesh] OR "Myocardial Infarction"[Mesh] OR "Stroke"[Mesh] OR "Cerebrovascular Disorders"[Mesh] OR "Peripheral Vascular Diseases"[Mesh] OR "Peripheral Arterial Disease"[Mesh] OR "Mortality"[Mesh] OR atherosclerosis OR arteriosclerosis OR "cardiovascular disease" OR "cardiovascular diseases" OR "myocardial infarction" OR stroke OR "cerebrovascular disease" OR "peripheral vascular disease" OR "peripheral arterial disease" OR mortality OR "blackfoot disease" OR "infarct*" OR "ischemia" OR "ischemic heart disease" OR "heart diseases" OR **[Body mass index and diabetes]** "obesity"[mh] OR "body mass index"[mh] OR "weight gain"[mh] OR "adipogenesis"[mh] OR "adipose tissue"[mh] OR "adipokines"[mh] OR "adiponectin"[mh] OR "leptin"[mh] OR resistin[mh]) OR ("diabetes mellitus"[mh] OR "glucose metabolism disorders"[mh] OR "insulin"[mh] OR "insulin resistance"[mh] OR "blood glucose"[mh] OR "islets of langerhans"[mh]) OR "body composition"

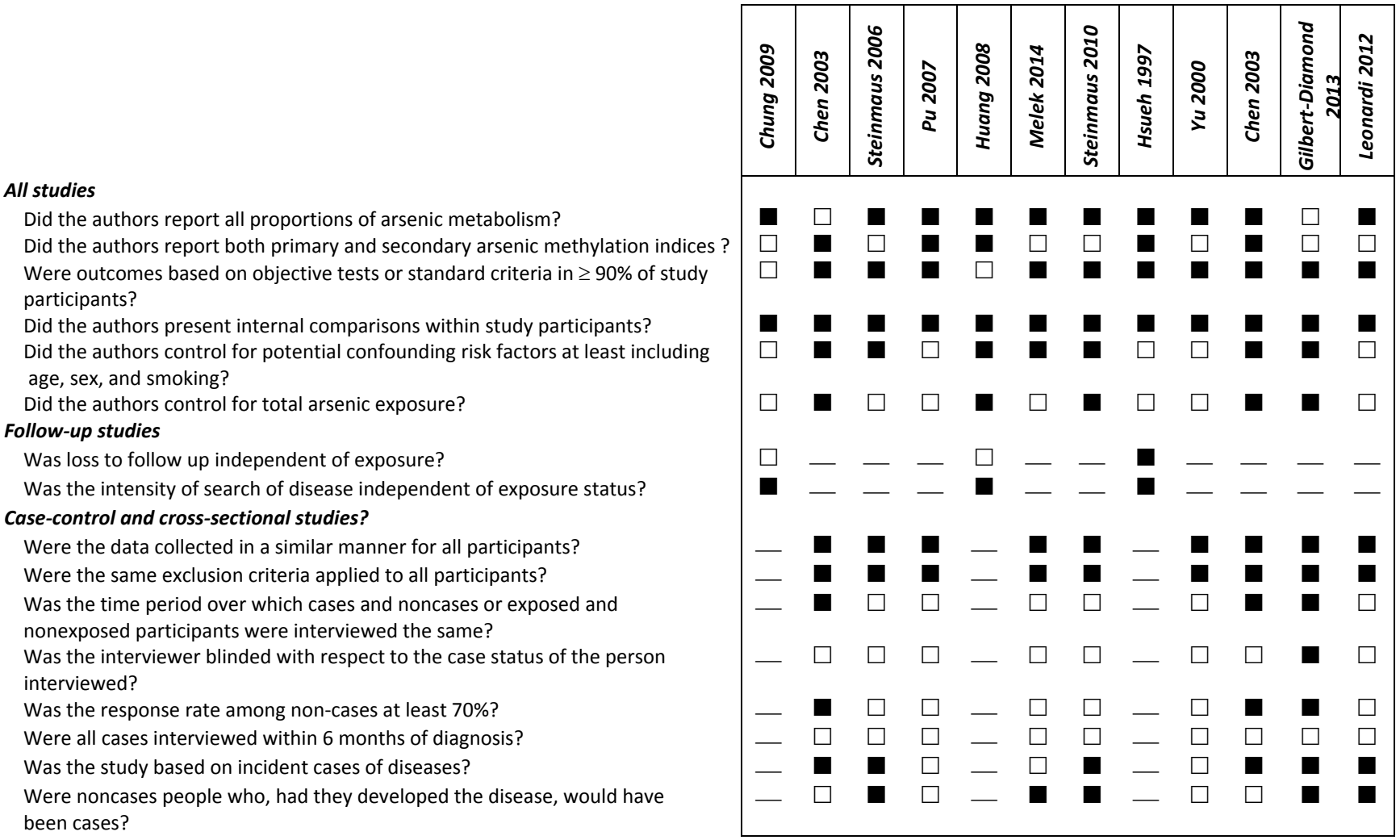
#4. Animal Study [3,874,066]	["animals"[MeSH Terms] NOT ("humans"[MeSH Terms] AND "animals"[MeSH Terms])]
Database	**EMBASE**
Date	April 3, 2014
Strategy	We combined the results for three major concepts(#1 to #3 below using "AND") with restriction to human studies (#4 using "NOT")
#1. Arsenic [18,740]	'arsenic'/exp OR 'arsenic poisoning'/exp OR arsenic AND poisoning OR 'arsenicals'/exp OR arsenicals OR arsenite OR arsenate
#2. Metabolism [7,482,077]	'metabolism'/exp OR metabolism OR 'methylation'/exp OR methylation OR 'arsenic methylatoin' OR 'arsenic metabolism'
#3. Health outcome measures [8,000,035]	**[Cancer]** 'cancer'/exp OR cancer OR 'neoplasm'/exp OR neoplasm OR 'malignancy' OR 'malignant' OR 'carotid artery diseases'/exp OR 'carotid artery diseases' OR 'coronary artery disease'/exp OR 'coronary artery disease' OR 'cardiovascular diseases'/exp OR **[Cardiovascular diseases]** 'cardiovascular diseases' OR 'myocardial infarction' OR 'myocardial infarction'/exp OR stroke OR 'stroke'/exp OR 'stroke' OR 'cerebrovascular disorders' OR 'cerebrovascular disorders'/exp OR 'peripheral vascular diseases' OR 'peripheral vascular diseases'/exp OR 'peripheral arterial diseases' OR 'peripheral arterial diseases'/exp OR 'mortality' OR 'mortality'/exp OR 'atherosclerosis'/exp OR 'atherosclerosis' OR atherosclerosis OR 'arteriosclerosis'/exp OR 'arteriosclerosis' OR arteriosclerosis OR 'blackfoot disease'/exp OR 'blackfoot disease' OR 'infarct'/exp OR 'infarct' OR infarct OR 'ischemia'/exp OR 'ischemia' OR ischemia OR 'ischemic heart disease'/exp OR 'ischemic heart disease' OR 'heart disease'/exp OR 'heart disease' OR **[Body mass index and diabetes]** 'diabetes' OR 'diabetes'/exp OR diabetes OR 'obesity' OR 'obesity'/exp OR obesity OR 'body mass index'/exp OR 'body mass index' OR 'weight gain'/exp OR 'weight gain' OR 'adipogenesis' OR 'adipogenesis'/exp OR adipogenesis OR 'adipose tissue'/exp OR 'adipose tissue' OR 'adipokines' OR 'adipokines'/exp OR adipokines OR 'adiponectin' OR 'adiponectin'/exp OR adiponectin OR 'leptin' OR 'leptin'/exp OR leptin OR 'resistin' OR 'resistin'/exp OR resistin OR 'glucose metabolism disorders'/exp OR 'glucose metabolism disorders' OR 'insulin' OR 'insulin'/exp OR insulin OR 'insulin resistance'/exp OR 'insulin resistance' OR 'blood glucose'/exp OR 'blood glucose' OR 'islets of langerhans'/exp OR 'islets of langerhans' OR 'body composition'/exp OR 'body composition'
#4. Animal Study	'animal'/exp NOT ('animal'/exp AND 'human'/exp)

[4,289,239]	

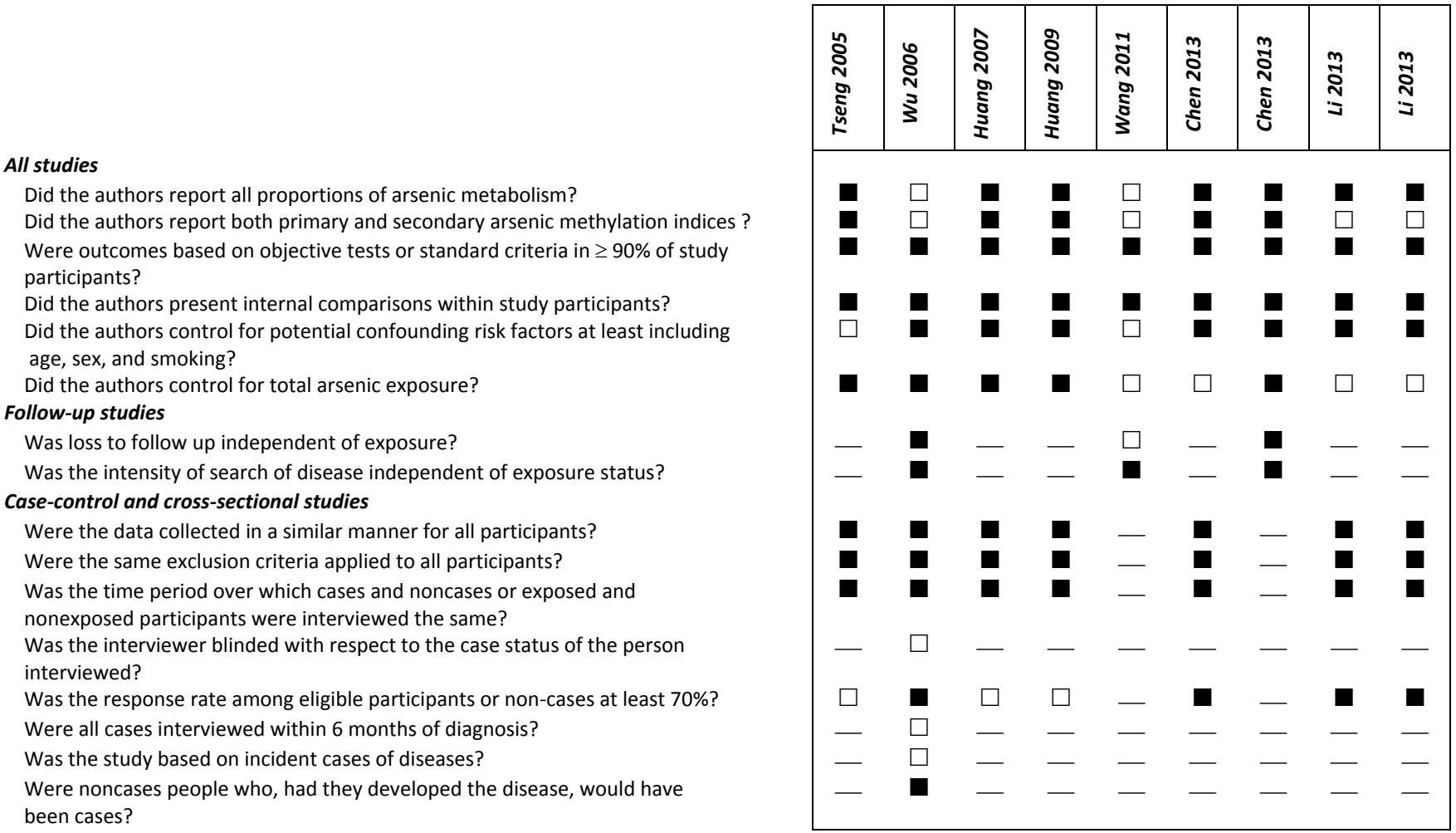
Supplementary figure 1. Flow diagram of study selection process.



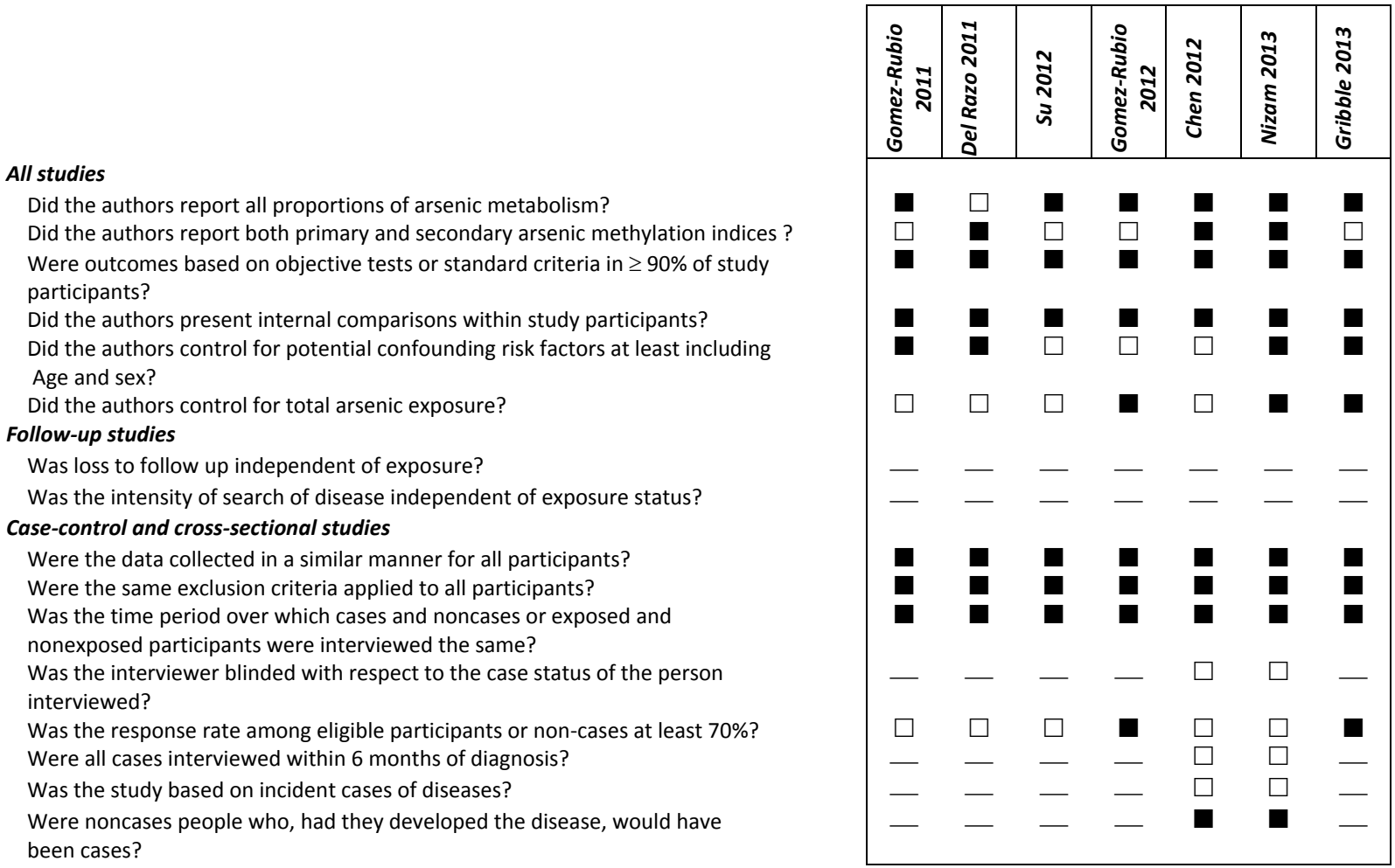
Supplementary figure 2. Quality criteria applied and evaluation of the design and data analysis issued in selected studies on the relation between arsenic metabolism and cancers.



Supplementary figure 3. Quality criteria applied and evaluation of the design and data analysis issued in selected studies on the relation between arsenic metabolism and cardiovascular diseases.



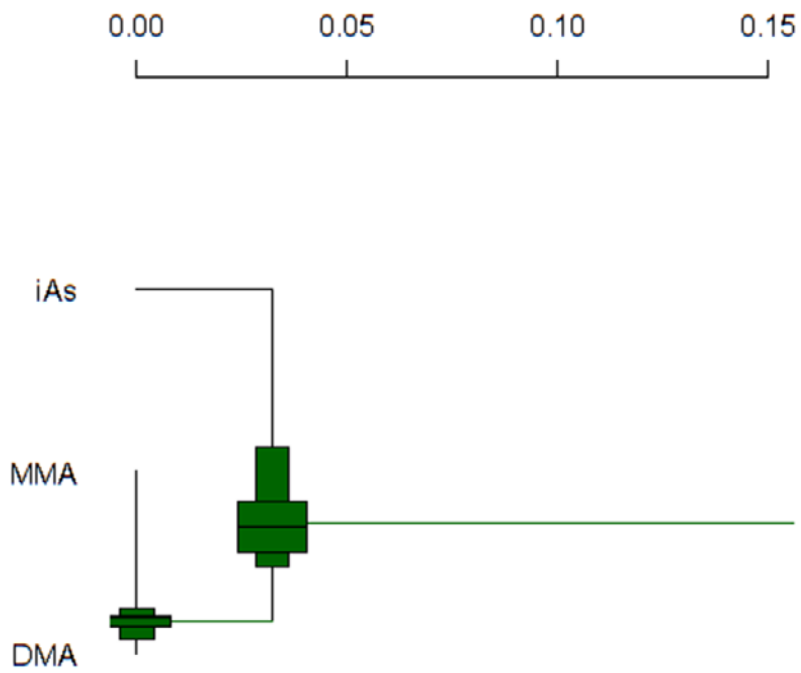
Supplementary figure 4. Quality criteria applied and evaluation of the design and data analysis issued in selected studies on the relation between arsenic metabolism and adiposity/diabetic phenotypes.



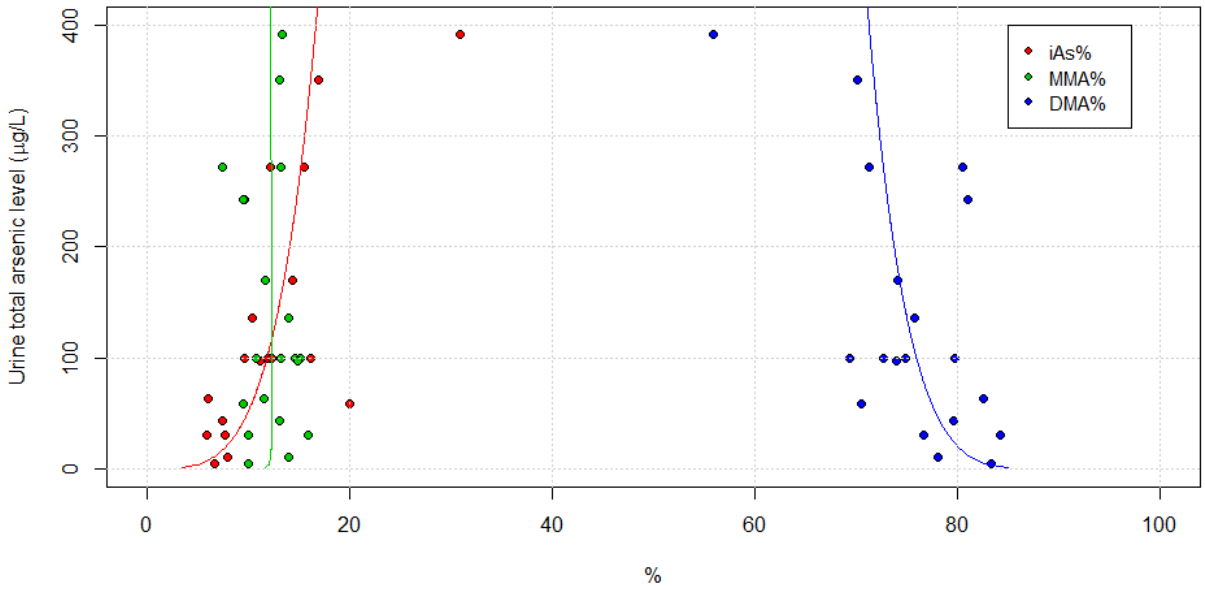
Supplementary table 2. Variation array of the analyzed compositions of arsenic metabolism from 19 studies.

	iAs%	MMA%	DMA%	
iAs%	—	0.21	0.27	Log-ratio variance
MMA%	1.09	—	0.06	
DMA%	6.78	6.22	—	
	Mean Compositional Ratio			
Compositional mean	11.3	12.3	76.4	

Supplementary figure 5. Coda-dendrogram of the isomeric log-ratio(ilr) basis for the compositions of arsenic metabolism. The horizontal green lines are proportional to the variance of each balance and the largest variance corresponds to the first balance comparing the inorganic arsenic and methylated arsenic species (MMA+DMA). The first and second balances (green boxes) point out larger presence of methylated arsenic species and DMA, respectively.



Supplementary figure 6. The regression lines between estimated urine total arsenic levels and compositional components of arsenic metabolism.



CHAPTER 2: The prospective association of arsenic metabolism with all-cause, cardiovascular and cancer mortality in the Strong Heart Study

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Suggested Key words: American Indians, arsenic, arsenic species, arsenic methylation, arsenic metabolism, mortality, cardiovascular disease, cancer, Strong Heart Study

Abstract

Context: The role of arsenic metabolism in mortality remains unclear and relevant evidence is scarce.

Objective: To assess the prospective association between arsenic metabolism and all-cause, cardiovascular, and cancer mortality in American Indian population exposed to low-moderate levels of arsenic

Design, Setting, and Participants: Prospective cohort study in 3,600 American Indian participants aged 45 to 75 years living in Arizona, Oklahoma, and North and South Dakota. The sum of urine inorganic arsenic (arsenite and arsenate), monomethylated (MMA), and dimethylated (DMA) arsenic compounds at baseline was used as the biomarker of inorganic arsenic exposure from multiple sources. The proportions of urine inorganic arsenic (arsenite and arsenate, iAs), MMA and DMA over the sum of inorganic and methylated species, expressed as iAs%, MMA%, and DMA%, was used to evaluate arsenic metabolism.

Main outcome measures: All-cause, cardiovascular, and cancer mortality. Causes of death were determined by the Strong Heart Study Mortality Review Committee.

Results: The median (interquartile range) for inorganic arsenic%, MMA% and DMA% was 8.0 (5.6 to 11.0), 14.0 (10.8 to 17.6) and 77.7 (71.9 to 82.6), respectively. The adjusted hazard ratio of all-cause mortality for an interquartile change increase in DMA% was 1.16 (95% CI 1.01-1.33) when it substituted iAs% whereas MMA% did not explain the risk of all-cause mortality. For cardiovascular mortality, the adjusted hazard ratio for an interquartile change increase in MMA% was 1.52 (1.16-1.99) and 1.17(1.01-1.35) when it substituted iAs% and DMA%, respectively. For cancer mortality, the adjusted

hazard ratio for an interquartile increase in MMA% was 0.73 (0.55-0.98) and 0.81 (0.67-0.97) when it substituted iAs% and DMA%, respectively.

Conclusion: Different patterns of arsenic metabolism profile are significantly associated with all-cause, cardiovascular and cancer mortality and the effects are independent of levels of arsenic exposure. More experimental and epidemiological evidence are needed to acquire more in-depth insights into the biological and clinical meaning of arsenic metabolism.

Background

Inorganic arsenic exposure is a major public health problem worldwide.[1] Indeed, chronic exposure to inorganic arsenic through water and foods has been associated with diverse chronic diseases including various forms of cancer[2], cardiovascular diseases[3, 4], diabetes[5, 6], and kidney dysfunction[7] at a wide range of arsenic exposure levels.

Chronic arsenic exposure has also been related to increased mortality, including all-cause[8], cancer[9, 10], and cardiovascular disease mortality[10-13] in many parts of the world including Argentina, Bangladesh, Chile, Taiwan, and the USA. Most studies used arsenic concentrations in well water or individual urine total arsenic concentrations as primary exposure matrices. Few studies, however, 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% inorganic arsenic [iAs], 10-20% monomethylarsonate [MMA], and 60-80% dimethylarsinate [DMA], with substantial inter-individual variation.[14, 15] Higher MMA% and lower DMA% in urine have been related to increased risk of various cancers[16, 17] and cardiovascular diseases,[18, 19] although some studies showed no or inconsistent association. In addition, recent studies have connected increased urine DMA% with increased prevalence of diabetes[20] and adiposity.[21] Possible mechanisms underlying a differential role for arsenic methylation patterns on disease outcomes could be related to one-carbon metabolism and methylation dysregulation.[22, 23] Understanding how arsenic methylation capacity is associated with mortality risks and whether the association is different by cause of death could be useful to arsenic risk assessment as well as to increase our understanding of arsenic toxicity mechanisms.

In this study, we examined the association of arsenic exposure and arsenic metabolism with the risk of mortality, including all-cause, cardiovascular and cancer mortality, in the Strong Heart Study, a large population-based prospective cohort with almost 20 years of follow-up.[12, 24] We also evaluated whether the association between arsenic metabolism and mortality was beyond the association with arsenic exposure levels.

Methods

Study population

The Strong Heart Study is a population-based cohort study that examined risk factors of cardiovascular mortality and morbidity in American Indians from Arizona, Oklahoma and North and South Dakota. Overall, 4549 men and women aged 45-74 years of age were enrolled between 1989 and 1991.[24] All eligible individuals were invited to participate in Arizona and Oklahoma, whereas a cluster sampling procedure was applied in North and South Dakota.[24, 25] The overall participation rate was 62%.[25] The study population was stable during the follow-up period due to low migration rates and strong cultural and community links among SHS participants.[26] Compared with nonparticipants, participants were similar in age, body mass index, and prevalence of self-reported diabetes but were more likely to be female and to have self-reported hypertension.[25] 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 available for arsenic measurements at the baseline visit. We then excluded 228 participants with some arsenic species data (inorganic arsenic, MMA or DMA) below the limit of detection, as arsenic metabolism cannot be evaluated at undetectable arsenic exposure levels, 5 missing smoking status, 2 missing education, 8 missing alcohol drinking status, 16 missing body mass index, 26 missing waist-hip ratio, 15 missing hypertension status, 66 missing estimated glomerular filtration rate, and 7 missing baseline fasting glucose level, leaving 3,600 participants for this analysis. Included participants were similar to those who were excluded because of missing data (data not shown).

Data collection

Baseline clinical information included a personal interview, physical examination, fasting blood test, and spot urine sample collection.[24] Socidemographic (age, sex, and education) and lifestyle (smoking and alcohol status) information was collected by trained and certified interviewers using standardized questionnaires.[24] Physical examination measurements (height, weight, waist and hip circumferences, and systolic and diastolic pressures) and bio-specimen collection (blood and urine) were conducted by centrally trained nurses and medical assistants following a standardized protocol.[24] Detailed procedures of clinical and laboratory examinations have been published.[24] Participants were asked to fast for 12 hours before blood samples were collected in the morning, at baseline and in the two subsequent visits. Serum creatinine was measured by an alkaline-picrate rate method.[24] Estimated glomerular filtration rate at baseline was derived from the 4-variable isotope dilution mass spectrometry Modification of Diet in Renal Disease Study equation.[27] Spot urine samples were collected in the morning and

were frozen with 1 to 2 hours of collection. The biospecimens were stored at -70°C or lower before analyses.[24] 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.[24]

Urine arsenic measurements

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), reflecting population stability and the appropriateness of one single urine arsenic sample to represent long-term arsenic exposure.[28] Detailed analytic methods and associated quality control procedures for arsenic analysis have been described.[29] Arsenic species concentrations were determined by high-performance liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS) that served as the arsenic selective detector (Agilent 1100 HPLC and Agilent 7700x ICP-MS, Agilent Technologies, Santa Clara, California). Arsenic speciation could discriminate species directly related to inorganic arsenic exposure (arsenite, arsenate, monomethylarsonate [MMA], and dimethylarsinate [DMA]) from those related to organic arsenicals in seafood (arsenobetaine as an overall marker of seafood arsenicals), which are generally considered nontoxic.[30] The limit of detection (LOD) for total arsenic and for inorganic arsenic (arsenite plus arsenate), MMA, DMA, and arsenobetaine plus other arsenic cations was 0.1 μg/L. The percentages of participants with concentrations below the limit of detection were 0.03% for total arsenic, 5.2% for inorganic arsenic, 0.8% for MMA, 0.03% for DMA, and 2.1% for arsenobetaine plus other arsenic cations. Levels of arsenic species below the limit of detection were replaced by the corresponding limit of detection

divided by the square root of 2. An in-house reference urine and the Japanese National Institute for Environmental Studies No. 18 Human Urine were analyzed together with the samples. Interassay coefficients of variation for total arsenic, inorganic arsenic, MMA, DMA and arsenobetaine plus other arsenic cations for the in-house reference urine were 4.4%, 6.0%, 6.5%, 5.9%, and 6.5%, respectively.

Arsenic exposure and arsenic metabolism

We used the sum of urine inorganic arsenic (arsenite and arsenate) and methylated arsenic species (MMA and DMA) as the biomarker of inorganic arsenic exposure from multiple sources.[31-33] Urine arsenic concentrations were divided by urine creatinine concentrations to account for urine dilution-concentration and expressed as μg/g creatinine. Urine concentrations of arsenobetaine and other arsenic cations were very low (median, 0.68; interquartile range, 0.41 to 1.54 μg/g creatinine), confirming that seafood intake was low in this sample, and indicating that DMA mainly came from inorganic arsenic exposure.[34]

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%, to evaluate arsenic metabolism.

Mortality follow-up

Vital status and cause-of-death codes were determined through 2008 by annual contact, review of hospitalization records and death certificates, and information obtained from the National Death Index. Mortality follow up was complete in 99.8% of the study participants. Study participants were followed from the date of the baseline examination

until the date of death or 31 December 2008, whichever occurred first. Cause of death were classified using the International Classification of Diseases, Ninth Revision (ICD-9) and were grouped into 4 broad categories by the SHS Mortality Review Committee based on the standardized mortality surveillance procedures including discharge summary of the terminal hospital admission, medical reports, autopsy, and pathology report (if available): cardiovascular diseases, cancer, respiratory and infectious disease, and all other causes. For cardiovascular disease deaths, the ascertainment of the specific cause of death was made through a central adjudication committee. Detailed definitions of the criteria used by the central adjudication committee have been described previously.[35]

Statistical analyses

Urine concentrations of the sum of inorganic and methylated species were modeled as quartiles and as log-transformed concentrations, comparing an interquartile range. Arsenic metabolism (iAs%, MMA%, and DMA%) was modeled as continuous, comparing an interquartile range.

We used Cox proportional hazards modeling to quantify the relative hazard of mortality associated with arsenic exposure and arsenic metabolism.[36] The time scale for survival analysis was age, 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). All proportional hazards models were adjusted for study sites (using the stratified Cox procedure), 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 (continuous), and waist-hip ratio (continuous). Although we

did not adjust for health conditions that could be in the causal pathway such as hypertension, and diabetes, we conducted stratified analysis to explore the consistency of the association between arsenic metabolism and mortality across levels of these comorbidities. We will also examined whether the association between arsenic metabolism and risk of mortality varied by sex, smoking status, body mass index (<25, 25-30, >30 kg/m^2), abdominal obesity (defined by waist circumference >112 cm and >88 cm for men and women, respectively).

All statistical analyses were performed in Stata/IC, version 12 (StataCorp, College Station, Texas) and with R, version 3.0.0 (R foundation for Statistical Computing, Vienna, Austria [www.r-project.org]).

Results

Study population

A total of 1,559 (43.3%) participants died of any cause over 51,810.3 person-years of follow-up; 484(13.4 %) died of cardiovascular disease (CVD), and 281 (7.8%) died of cancer. Overall, median concentration of the sum of inorganic and methylated arsenic species in the urine was 11.2 μg/L (interquartile range, 6.6 to 19.1 μg/L). Urine arsenic concentrations were higher in participants from Arizona (median 14.9 μg/L), followed by the Dakotas (12.6 μg/L) and Oklahoma (median 7.2 μg/L). The median (interquartile range) for iAs%, MMA% and DMA% was 8.0 (5.6 to 11.0)%, 14.0 (10.8 to 17.6)% and 77.7 (71.9 to 82.6)%, respectively.

Arsenic exposure and all-cause and cause specific mortality

Baseline urine concentrations of inorganic arsenic, methylated arsenic species including MMA and DMA, and the sum of inorganic and methylated arsenic species were significantly higher among participants who died during the follow-up (Table 1). The fully adjusted hazard ratios for all-cause mortality, CVD mortality, and cancer mortality were 1.28 (95% CI 1.16-1.41), 1.28 (1.08-1.52), and 1.15 (0.92-1.44), respectively, for an interquartile range increase in urine concentrations of the sum of inorganic and methylated arsenic species (Table 2).

Arsenic metabolism and all-cause mortality

Before adjustment, baseline arsenic metabolism profiles were comparable between survivors and deceased participants (Table 1). When modeling each arsenic metabolism biomarker one at a time, each interquartile range increase in iAs%, MMA%, and DMA% were prospectively associated with all-cause mortality with hazard ratio 0.91 (95%CI 0.85 -0.97), 0.91 (CI 0.85-0.98), and 1.12 (1.04-1.21) in fully adjusted models, respectively (Table 3, model 3). When modeling arsenic metabolism by including two biomarkers at the same time, the adjusted hazard ratio of mortality for an interquartile range increase in iAs% was 0.97 (95% CI 0.87-1.09) when it substituted MMA% and 0.93 (0.87-0.99) when it substituted DMA%.

The adjusted hazard ratio of mortality for an interquartile range increase in MMA% was 1.03 (95% CI 0.90-1.19) and 0.94 (0.87-1.03) when it substituted iAs% and DMA%, respectively. The adjusted hazard ratio of mortality for an interquartile range increase in DMA% was 1.16 (1.01-1.33) and 1.10 (0.96-1.25) when it substituted iAs%

and MMA%, respectively (Table 3, model 3). In dose-response analyses, increasing DMA% was related to increased all-cause mortality when it substituted both iAs% and MMA% (Figure 1). iAs% was associated with lower all-cause mortality when it substituted DMA% but not when it substituted MMA%. The association between arsenic metabolism and all-cause mortality was stronger in participants with female gender, diabetes, and obesity. However, the association was similar across all arsenic exposure categories (Table 6).

Arsenic metabolism and cardiovascular disease mortality

When including each arsenic metabolism biomarker in the multivariable Cox proportional hazards model one at a time, the fully adjusted hazard ratio of cardiovascular diseases (CVD) mortality for each interquartile range increase in iAs%, MMA%, and DMA% was 0.86 (95%CI 0.76 -0.97), 1.05 (CI 0.93-1.20), and 1.07 (0.94-1.21), respectively (Table 4, model 3). When modeling arsenic metabolism by including two biomarkers in the Cox regression model at the same time, the adjusted hazard ratio for an interquartile range increase in iAs% was 0.72 (95% CI 0.58-0.89) and 0.81 (0.71-0.93) when it substituted MMA% and DMA%, respectively. The adjusted hazard ratio for an interquartile range increase in MMA% was 1.52 (95% CI 1.16-1.99) and 1.17 (1.01-1.35) when it substituted iAs% and DMA%, respectively. The adjusted hazard ratio for an interquartile range increase in DMA% was 1.53 (1.16-2.00) and 0.78 (0.63-0.98) when it substituted iAs% and MMA%, respectively (Table 4, model 3). In dose-response analyses, increasing MMA% was related to increased CVD mortality when it substituted both% inorganic arsenic and DMA% (Figure 2). DMA% was associated with increased CVD mortality when it substituted iAs% but not when it substituted MMA%. The

association between arsenic metabolism and cardiovascular mortality was similar across all arsenic exposure categories (Table 7).

Arsenic metabolism and cancer mortality

When including each arsenic metabolism biomarker in the modeling one at a time, the hazard ratio of cancer mortality for each interquartile range increase in iAs%, MMA%, and DMA% was 1.02 (95%CI 0.89 -1.17), 0.84 (CI 0.70-1.00), and 1.09 (0.92-1.29) in full adjustment models, respectively (Table 5, model 3). When modeling arsenic metabolism by including two biomarkers in the Cox regression model at the same time, the adjusted hazard ratio for an interquartile range increase in iAs% was 1.28 (95% CI 1.02-1.62) and 1.08 (0.95-1.24) when it substituted MMA% and DMA%, respectively. The adjusted hazard ratio for an interquartile range increase in MMA% was 0.73 (95% CI 0.55-0.98) and 0.81 (0.67-0.97) when it substituted iAs% and DMA%, respectively. The adjusted hazard ratio for an interquartile range increase in DMA% was 0.85 (0.65-1.12) and 1.40 (1.04-1.87) when it substituted iAs% and MMA%, respectively (Table 5, model 3). In dose-response analyses, increasing MMA% was related to lower cancer mortality when it substituted both% inorganic arsenic and DMA% (Figure 3). DMA% was associated with increased cancer mortality when it substituted MMA% but not when it substituted iAs%. The association between arsenic metabolism and cardiovascular mortality was similar across all arsenic exposure categories (Table 8).

Discussion

Research on the role of arsenic metabolism in all-cause mortality and cause-specific mortality is scarce. Our study is the first study to systematically examine the relationship between arsenic metabolism and mortality using data from a population-based cohort. We found the substitution of iAs% by DMA% was prospectively associated with higher all-cause mortality. The substitution of iAs% by either MMA% or DMA% was associated with higher cardiovascular disease (CVD) mortality. The substitution of DMA% by MMA% was also related to higher CVD mortality. For cancer mortality, the substitution of MMA% by either iAs% or DMA% was prospectively associated with higher cancer mortality.

The mechanism underlying the association between arsenic metabolism and all-cause mortality remains unclear though many biological hypotheses have been raised.[22] One of the major hypothesis 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.[37] The optimal balance between nutrition and one-carbon metabolism is critical to maintain genome stability, modulate epigenomics, and keep cellular homeostasis and detoxification. Metabolic imbalance from methylation dysregulation in one-carbon metabolism has been specially linked to the development of cancer, cardiovascular diseases, and diabetes, which could potentially explain the arsenic-related pleiotropic adverse effect. To what extent arsenic interferes with one-carbon metabolism remains to be determined; however, inter-individual variation in arsenic methylation profile may reflect both differential individual susceptibility toward arsenic

exposure and differential metabolic capacity to maintain methyl balance, the fundamental driver of various downstream physiologic reactions.[38] Increasing evidence has shown that nutrition (e.g. folic acid supplementation) can play a role in mitigating arsenic toxicity.[39] Arsenic exposure has also been associated with global DNA methylation in a number of studies, although studies targeting on arsenic metabolism and epigenomic patterns is limited.[40-42] Our findings will motivate experimental and clinical research to investigate the biological mechanisms and potential interventions for adjusting arsenic metabolism in risk modification and risk reduction in arsenic-related health problems.

Strengths of the current study include careful modeling of the dynamic of arsenic metabolism, standardized protocol to ascertain mortality data over a 20-year follow-up and high-quality laboratory methods for measuring concentrations of urine arsenic species. This study had several limitations. First, the urine arsenic concentrations and metabolism were measured in a single sample at baseline to represent internal doses and individual metabolism profiles. However, we have confirmed that arsenic levels in urine and arsenic metabolism were constant over 10 years in this population.[28] Second, over-adjustment for variables that possibly in the cause pathway (e.g. HbA1c and fasting glucose in all-cause mortality) could not be excluded. However, multiple sensitively analyses yielded consistent results. Third, it is possible that unknown and unmeasured confounding may bias our findings. For example, we do not have all possible measurements of human exposure to environmental toxicants that may modify both arsenic metabolism profiles and risk of mortality. Finally, given the observational nature of this study, we cannot firmly conclude that the association between arsenic metabolism and all-cause and cause-specific mortality reflects cause and effect.

Conclusion

This is the first study to show that specific profiles of arsenic metabolism are associated with all-cause, cardiovascular disease, and cancer mortality. The patterns were different for cardiovascular and cancer mortality. Understanding the differential individual susceptibility measured by arsenic metabolism to the risk of mortality can be critical in risk assessment of arsenic toxicity. Additional experimental and epidemiological evidence are needed to understand the biological reasons and clinical implications of arsenic metabolism.

Table 1. Characteristics of Strong Heart Study participants at baseline (1989-1991).

	Survivors n=2,041, 56.7%		**Deaths n=1,559, 43.3%**		***p*-value**
	N (%)	**Median(IQR)**	**N %**	**Median(IQR)**	
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
Location					<0.01
Arizona	633 (31.0)		630 (40.4)		
Oklahoma	747 (36.6)		370 (23.7)		
North and South Dakota	661 (32.4)		559 (35.9)		
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		30.4 (27.1-34.6)		29.7 (26.0-33.9)	<0.01
Waist-hip ratio		0.95 (0.91-0.98)		0.96 (0.93-1.00)	<0.01
Waist circumference (cm)		104 (96-115)		104 (95-114)	0.81
% Body fat		37.8 (30.4-43.9)		34.5 (28.2-42)	<0.01
Urine creatinine, g/L		1.28 (0.83-1.79)		1.11 (0.73-1.61)	<0.01
Specific gravity		1.02 (1.015-1.024)		1.018 (1.014-1.023)	0.27
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, %	N=1,922	5.4 (4.9-6.6)		6.1 (5.1-9.2)	<0.01
Arsenic exposure					
iAs + methylated arsenic*, 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
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

Table 2. Hazard ratios (95% confidence intervals) for all-cause, cardiovascular, and cancer mortality per interquartile range in urine concentrations of inorganic arsenic (iAs), monomethylarsonate (MMA), dimethylarsinate (DMA) and the sum of iAs, MMA and DMA (µg/g creatinine).

Arsenic (interquartile range)	Model 1	Model 2	Model 3 (urine creatinine)	Model 4 (Specific gravity)	Model 5 (No dilution adj.)
All-cause mortality			**N=3,404**	**N=3,404**	**N=3,404**
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)
iAs + methylated arsenic (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 (CVD) mortality			**N=3,542**	**N=3,542**	**N=3,542**
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)
iAs + methylated arsenic (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			**N=3,571**	**N=3,571**	**N=3,571**
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)
iAs + methylated arsenic (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 CVD 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 ratios (95% confidence intervals) 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 biomarkers at a time. All models adjusted for the urine concentrations of sum of iAs, MMA and DMA corrected by urine creatinine except model 4 and 5.

Arsenic metabolism (interquartile range)	Model 1	Model 2	Model 3 (Urine creatinine) (N=3,404)	Model 4 (Specific gravity) (N=3,404)	Model 5 (No dilution adj.) (N=3,404)
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 biomarker 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

Figure 1. Hazard ratios for all-cause mortality 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). The shaded areas represent 95% CIs.

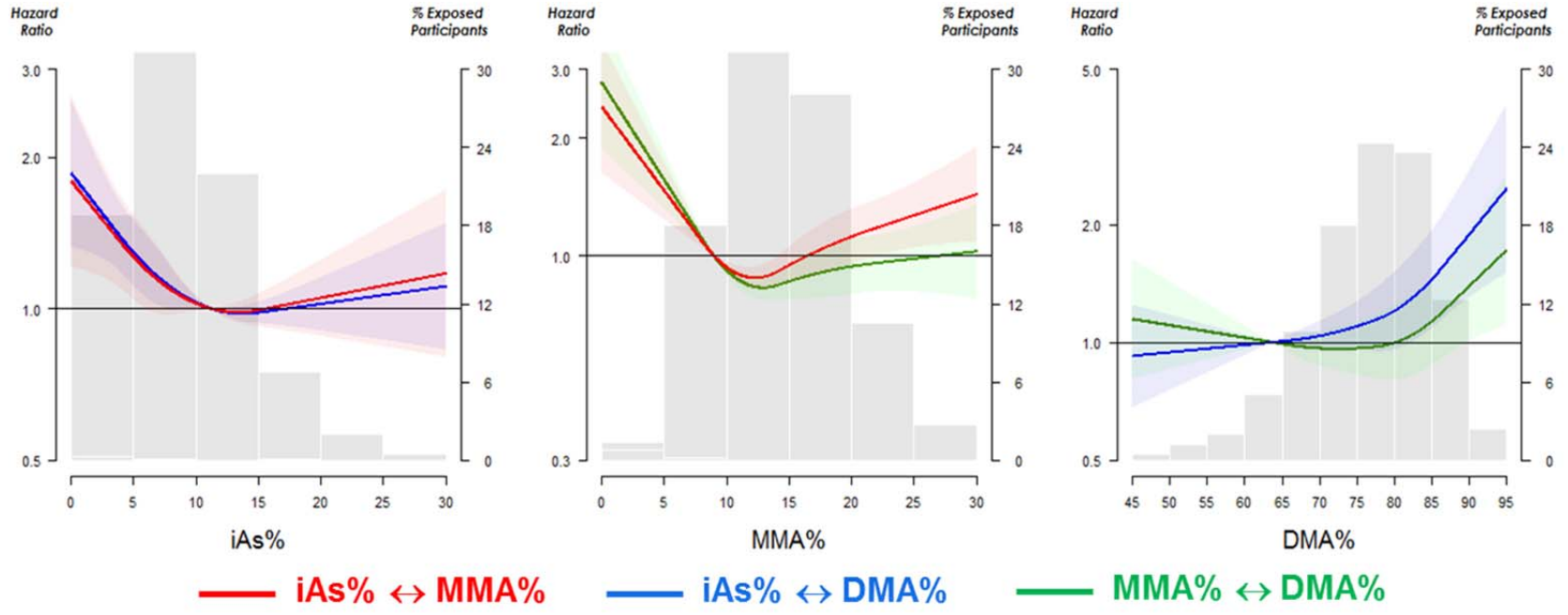

Table 4. Hazard ratios (95% confidence intervals) for cardiovascular mortality per interquartile range in arsenic metabolism biomarkers (inorganic arsenic% [iAs%], monomethylarsonate% [MMA%] and dimethylarsinate% [DMA%]). As the three biomarkers equal 100%, models entered two biomarkers at a time. All models adjusted for the urine concentrations of sum of iAs, MMA and DMA corrected by urine creatinine except model 4 and 5.

Arsenic metabolism (interquartile range)	Model 1	Model 2	Model 3 (Urine creatinine) (N=3,542)	Model 4 (Specific gravity) (N=3,542)	Model 5 (No dilution adj.) (N=3,542)
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 biomarker 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-trasnformed), 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, LDL, 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

Figure 2. Hazard ratios for cardiovascular mortality 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). The shaded areas represent 95% CIs.

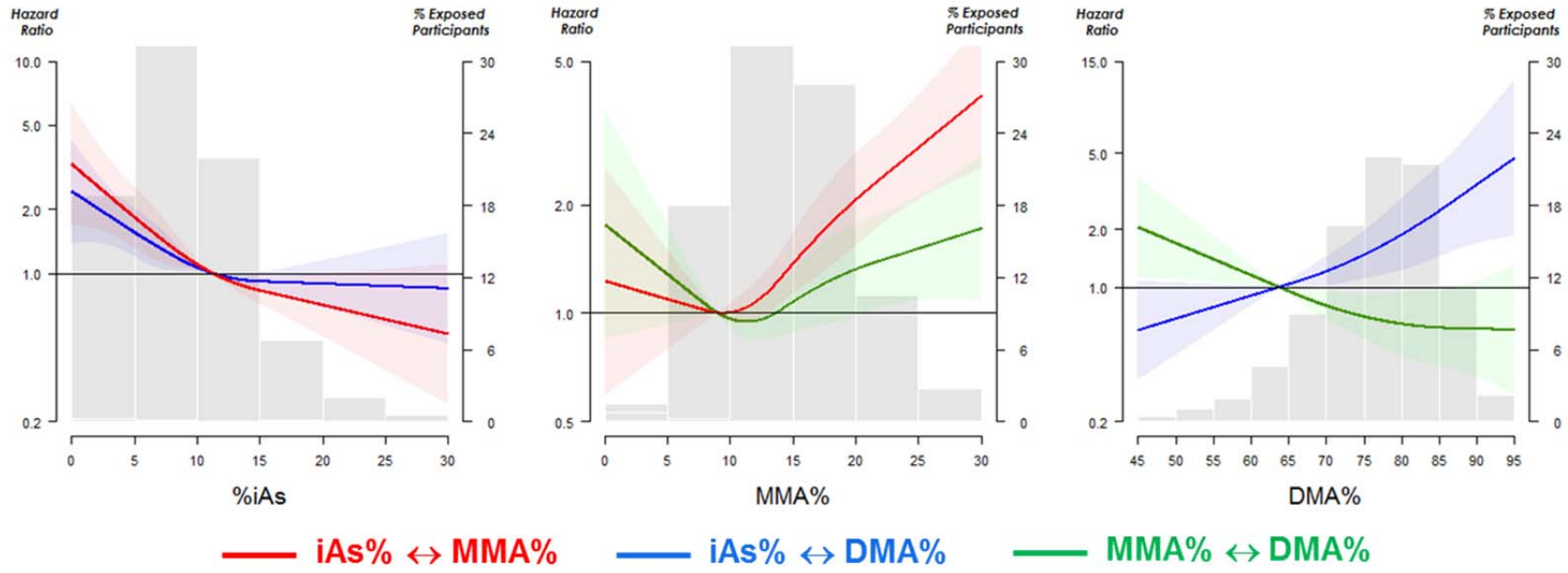

Table 5. Hazard ratios (95% confidence intervals) for cancer mortality per interquartile range in arsenic metabolism biomarkers (inorganic arsenic% [iAs%], monomethylarsonate% [MMA%] and dimethylarsinate% [DMA%]). As the three biomarkers equal 100%, models entered two biomarkers at a time. All models adjusted for the urine concentrations of sum of iAs, MMA and DMA corrected by urine creatinine except model 4 and 5.

Arsenic metabolism (interquartile range)	Model 1	Model 2	Model 3 (Urine creatinine) (N=3,571)	Model 4 (Specific gravity) (N=3,571)	Model 5 (No dilution adj.) (N=3,571)
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 biomarker 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

Figure 3. Hazard ratios for cancer mortality 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). The shaded areas represent 95% CIs.

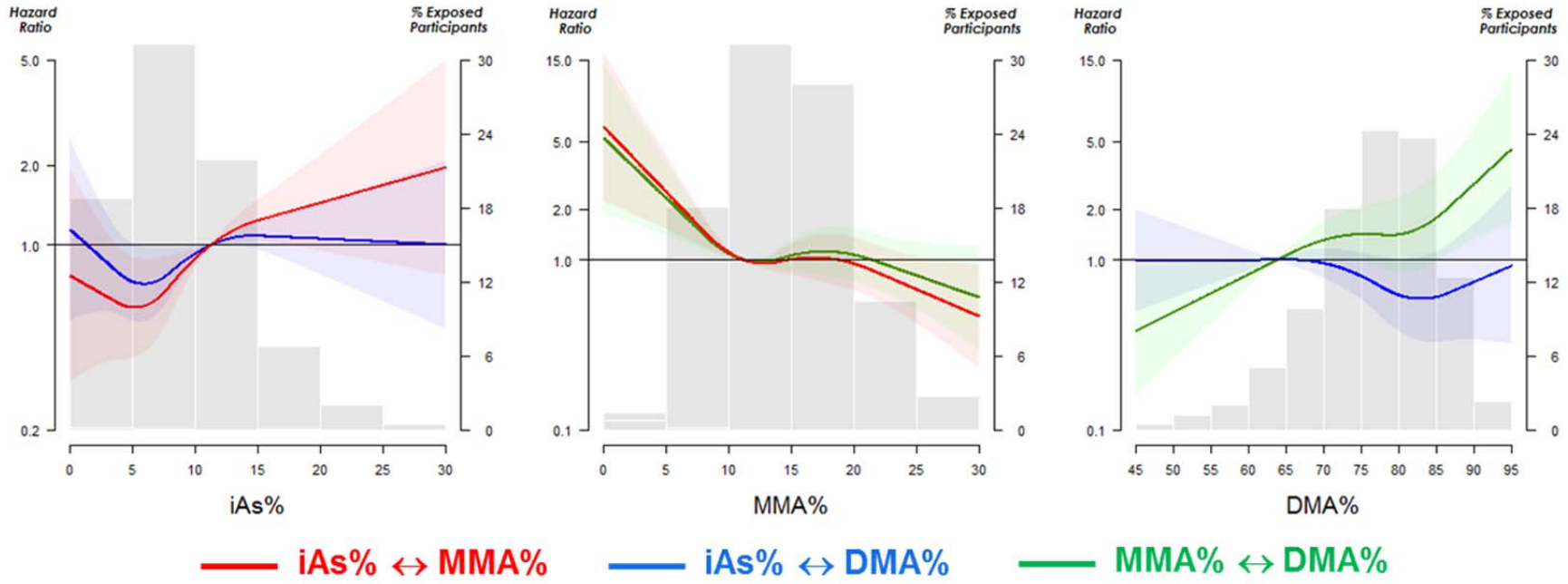

Table 6. Adjusted hazard ratios and 95% confidence intervals for all-cause mortality comparing the 75th with 25th percentile of inorganic arsenic%[iAs%], monomethylarsonate% [MMA%] and dimethylarsonate%[DMA%], by participant characteristics at baseline.

Subgroup	n	iAs% Substituted by		MMA% Substituted by		DMA% Substituted by	
		MMA%	DMA%	iAs%	DMA%	iAs%	MMA%
Age (years)							
< 55	1723	0.98 (0.77-1.25)	1.23 (0.97-1.55)	1.02 (0.84-1.23)	1.26 (1.01-1.58)	0.90 (0.80-1.02)	0.86 (0.75-0.99)
≥ 55	1681	1.05 (0.89-1.25)	1.09 (0.92-1.30)	0.96 (0.84-1.10)	1.01 (0.86-1.18)	0.96 (0.88-1.04)	1.00 (0.90-1.10)
p-value for interaction		0.71	0.69	0.71	0.82	0.69	0.82
Sex							
Men	1423	0.95 (0.79-1.15)	1.02 (0.85-1.21)	1.04 (0.90-1.21)	1.10 (0.93-1.32)	0.99 (0.91-1.08)	0.94 (0.84-1.05)
Women	1981	1.27 (1.00-1.18)	1.52 (1.18-1.96)	0.83 (0.68-1.00)	1.05 (0.86-1.28)	0.81 (0.71-0.92)	0.97 (0.86-1.10)
p-value for interaction		<0.01	<0.01	<0.01	0.22	<0.01	0.22
Study site							
Arizona	1230	1.23 (0.95-1.59)	1.43 (1.11-1.83)	0.85 (0.69-1.04)	1.03 (0.82-1.28)	0.84 (0.74-0.95)	0.98 (0.86-1.13)
Oklahoma	1023	0.92 (0.66-1.28)	0.96 (0.69-1.34)	1.07 (0.82-1.39)	1.10 (0.83-1.46)	1.02 (0.86-1.20)	0.94 (0.79-1.13)
North/South Dekota	1151	0.95 (0.77-1.16)	1.08 (0.89-1.30)	1.05 (0.89-1.23)	1.17 (0.96-1.44)	0.96 (0.88-1.06)	0.90 (0.79-1.03)
p-value for interaction		0.04	0.08	0.04	0.13	0.08	0.13
Smoking							
Never	1098	0.88 (0.70-1.09)	0.97 (0.79-1.19)	1.11 (0.93-1.32)	1.19 (0.94-1.52)	1.02 (0.92-1.13)	0.89 (0.77-1.04)
Former	1151	1.28 (0.98-1.69)	1.47 (1.11-1.94)	0.82 (0.66-1.02)	0.99 (0.78-1.25)	0.82 (0.72-0.95)	1.01 (0.87-1.17)
Current	1155	1.03 (0.81-1.31)	1.16 (0.93-1.46)	0.98 (0.81-1.19)	1.11 (0.89-1.39)	0.93 (0.83-1.04)	0.93 (0.81-1.07)
p-value for interaction		0.52	0.18	0.52	0.99	0.18	0.99
DM							
No	1722	0.78 (0.65-0.96)	0.90 (0.74-1.09)	1.21 (1.03-1.41)	1.30 (1.07-1.58)	1.06 (0.96-1.16)	0.85 (0.75-0.96)
Yes	1682	1.36 (1.11-1.67)	1.50 (1.22-1.83)	0.78 (0.67-0.92)	0.92 (0.77-1.09)	0.82 (0.74-0.90)	1.06 (0.94-1.18)
p-value for interaction		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Hypertension							
No	1702	0.92 (0.75-1.12)	1.05 (0.87-1.26)	1.07 (0.92-1.26)	1.21 (0.99-1.48)	0.98 (0.89-1.07)	0.89 (0.78-1.01)

Yes	1675	1.11 (0.90-1.35)	1.21 (0.99-1.48)	0.92 (0.79-1.08)	1.03 (0.87-1.22)	0.91 (0.82-1.01)	0.98 (0.88-1.09)
p-value for interaction		0.13	0.15	0.13	0.26	0.15	0.26
Obesity							
BMI< 30 kg/m^2	1678	0.92 (0.78-1.08)	1.01 (0.86-1.18)	1.07 (0.94-1.22)	1.16 (0.98-1.36)	1.00 (0.92-1.08)	0.91 (0.82-1.01)
BMI≥ 30	1726	1.32 (1.02-1.70)	1.55 (1.20-2.00)	0.80 (0.66-0.98)	1.00 (0.81-1.24)	0.80 (0.71-0.91)	1.00 (0.87-1.14)
p-value for interaction		0.04	<0.01	0.04	0.70	<0.01	0.70
Waist-hip ratio							
Non-abdominal obesity*	953	0.83 (0.67-1.04)	0.89 (0.73-1.09)	1.16 (0.97-1.37)	1.18 (0.96-1.46)	1.06 (0.96-1.17)	0.90 (0.79-1.03)
Abdominal obesity	2451	1.25 (1.02-1.52)	1.45 (1.19-1.78)	0.84 (0.72-0.98)	1.02 (0.87-1.21)	0.83 (0.75-0.92)	0.99 (0.89-1.10)
p-value for interaction		<0.01	<0.01	<0.01	0.21	<0.01	0.21
iAs+MMA+DMA							
≤ 5.5 μg/g	845	1.27 (0.84-1.91)	1.27 (0.82-1.98)	0.83 (0.60-1.14)	0.87 (0.64-1.19)	0.89 (0.71-1.10)	1.09 (0.90-1.32)
> 5.5 & ≤ 8.8 μg/g	842	1.25 (0.90-1.73)	1.28 (0.92-1.77)	0.84 (0.65-1.08)	0.89 (0.68-1.18)	0.89 (0.75-1.04)	1.07 (0.90-1.28)
>8.8 & ≤ 13.9 μg/g	850	0.80 (0.61-1.06)	1.04 (0.81-1.34)	1.19 (0.96-1.48)	1.47 (1.13-1.93)	0.98 (0.86-1.11)	0.78 (0.66-0.93)
> 13.9 μg/g	867	0.99 (0.79-1.25)	1.17 (0.93-1.45)	1.00 (0.84-1.21)	1.18 (0.94-1.48)	0.93 (0.83-1.03)	0.90 (0.78-1.04)
p-value for interaction		0.17	0.41	0.17	0.18	0.41	0.18
Overall	3404	1.03 (0.90-1.19)	1.16 (1.01-1.33)	0.97 (0.87-1.09)	1.10 (0.96-1.25)	0.93 (0.87-1.00)	0.94 (0.87-1.03)

Table 7. Adjusted hazard ratios and 95% confidence intervals for cardiovascular mortality comparing the 75th with 25th percentile of inorganic arsenic%[iAs%], monomethylarsonate% [MMA%] and dimethylarsonate%[DMA%], by participant characteristics at baseline.

Subgroup	n	iAs% Substituted by		MMA% Substituted by		DMA% Substituted by	
		MMA%	DMA%	iAs%	DMA%	iAs%	MMA%
Age (years)							
< 55	1780	1.62 (1.05-2.50)	1.72 (1.10-2.70)	0.68 (0.48-0.96)	0.80 (0.55-1.16)	0.76 (0.61-0.95)	1.15 (0.91-1.45)
≥ 55	1762	1.45 (1.04-2.03)	1.42 (1.01-1.99)	0.74 (0.57-0.97)	0.79 (0.59-1.04)	0.84 (0.71-0.99)	1.16 (0.97-1.39)
p-value for interaction		0.56	0.51	0.56	0.75	0.51	0.75
Sex							
Men	1470	1.46 (1.00-2.12)	1.37 (0.96-1.97)	0.74 (0.55-1.00)	0.75 (0.55-1.04)	0.85 (0.71-1.02)	1.19 (0.98-1.46)
Women	2072	1.91 (1.27-2.86)	2.05 (1.31-3.20)	0.60 (0.43-0.83)	0.74 (0.53-1.03)	0.70 (0.56-0.87)	1.21 (0.98-1.50)
p-value for interaction		0.27	0.21	0.27	0.61	0.21	0.61
Study site							
Arizona	1241	1.98 (1.30-3.03)	2.18 (1.37-3.49)	0.58 (0.41-0.81)	0.74 (0.52-1.05)	0.68 (0.53-0.86)	1.21 (0.97-1.51)
Oklahoma	1095	1.47 (0.81-2.64)	1.39 (0.75-2.56)	0.74 (0.46-1.18)	0.76 (0.47-1.21)	0.85 (0.62-1.15)	1.19 (0.89-1.61)
North/South Dekota	1206	1.16 (0.79-1.70)	1.18 (0.82-1.68)	0.89 (0.66-1.20)	0.93 (0.65-1.34)	0.92 (0.77-1.10)	1.05 (0.83-1.32)
p-value for interaction		0.02	0.03	0.02	0.10	0.03	0.10
Smoking							
Never	1136	1.28 (0.84-1.96)	1.27 (0.82-1.95)	0.82 (0.59-1.15)	0.85 (0.57-1.26)	0.89 (0.72-1.10)	1.11 (0.86-1.42)
Former	1194	2.46 (1.46-4.12)	2.20 (1.28-3.79)	0.49 (0.32-0.74)	0.53 (0.36-0.80)	0.67 (0.51-0.88)	1.49 (1.16-1.92)
Current	1212	1.17 (0.75-1.83)	1.36 (0.88-2.09)	0.88 (0.62-1.26)	1.06 (0.71-1.57)	0.86 (0.69-1.06)	0.97 (0.75-1.24)
p-value for interaction		0.11	0.13	0.11	0.17	0.13	0.17
Hypertension							
No	1791	1.42 (0.95-2.13)	1.28 (0.88-2.13)	0.76 (0.55-1.04)	0.74 (0.50-1.08)	0.88 (0.73-1.07)	1.21 (0.95-1.55)
Yes	1751	1.69 (1.20-2.38)	1.83 (1.27-2.63)	0.66 (0.50-0.86)	0.80 (0.60-1.05)	0.74 (0.62-0.89)	1.15 (0.97-1.37)
p-value for interaction		0.16	0.04	0.16	0.83	0.04	0.83
Obesity							
BMI< 30 kg/m^2	1743	1.29 (0.92-1.82)	1.26 (0.90-1.76)	0.82 (0.62-1.07)	0.84 (0.61-1.14)	0.89 (0.75-1.06)	1.12 (0.92-1.36)

BMI≥ 30	1799	1.83 (1.20-2.80)	1.87 (1.21-2.91)	0.62 (0.44-0.87)	0.72 (0.51-1.01)	0.73 (0.59-0.91)	1.23 (0.99-1.53)
p-value for interaction		0.29	0.20	0.29	0.66	0.20	0.66
Waist-hip ratio							
Non-abdominal obesity*	997	1.14 (0.71-1.82)	1.11 (0.71-1.74)	0.90 (0.62-1.31)	0.91 (0.60-1.36)	0.95 (0.76-1.19)	1.06 (0.82-1.38)
Abdominal obesity	2545	1.73 (1.24-2.42)	1.78 (1.25-2.53)	0.65 (0.50-0.84)	0.75 (0.57-0.98)	0.75 (0.63-0.89)	1.20 (1.01-1.43)
p-value for interaction		0.17	0.14	0.17	0.40	0.14	0.40
iAs+MMA+DMA							
≤ 5.5 μg/g	884	1.40 (0.72-2.72)	1.21 (0.58-2.51)	0.77 (0.45-1.30)	0.71 (0.44-1.16)	0.91 (0.63-1.31)	1.24 (0.91-1.69)
> 5.5 & ≤ 8.8 μg/g	888	1.12 (0.64-1.95)	1.15 (0.66-2.02)	0.91 (0.59-1.42)	0.97 (0.59-1.58)	0.93 (0.70-1.23)	1.02 (0.75-1.39)
>8.8 & ≤ 13.9 μg/g	887	1.36 (0.79-2.35)	1.52 (0.92-2.52)	0.78 (0.51-1.20)	0.93 (0.56-1.53)	0.81 (0.63-1.04)	1.05 (0.76-1.43)
> 13.9 μg/g	883	1.98 (1.26-3.11)	2.11 (1.29-3.44)	0.58 (0.41-0.83)	0.72 (0.49-1.05)	0.69 (0.54-0.88)	1.23 (0.97-1.57)
p-value for interaction		0.36	0.25	0.36	0.78	0.25	0.78
Overall	3404	1.52 (1.16-1.99)	1.53 (1.16-2.00)	0.72 (0.58-0.89)	0.78 (0.63-0.98)	0.81 (0.71-0.93)	1.17 (1.01-1.35)

Table 8. Adjusted hazard ratios and 95% confidence intervals for cancer mortality comparing the 75th with 25th percentile of inorganic arsenic%[iAs%], monomethylarsonate% [MMA%] and dimethylarsonate%[DMA%], by participant characteristics at baseline.

Subgroup	n	iAs% Substituted by		MMA% Substituted by		DMA% Substituted by	
		MMA%	DMA%	iAs%	DMA%	iAs%	MMA%
Age (years)							
< 55	1796	0.73 (0.41-1.30)	0.95 (0.55-1.63)	1.28 (0.81-2.01)	1.55 (0.89-2.68)	1.03 (0.78-1.35)	0.76 (0.54-1.07)
≥ 55	1775	0.72 (0.52-1.00)	0.80 (0.59-1.09)	1.30 (1.00-1.69)	1.35 (0.96-1.90)	1.12 (0.96-1.30)	0.83 (0.67-1.03)
p-value for interaction		0.62	0.99	0.62	0.44	0.99	0.44
Sex							
Men	1483	0.70 (0.47-1.05)	0.86 (0.59-1.25)	1.33 (0.96-1.83)	1.51 (1.01-2.27)	1.08 (0.89-1.30)	0.77 (0.60-0.99)
Women	2088	0.78 (0.50-1.21)	0.87 (0.57-1.34)	1.22 (0.86-1.73)	1.29 (0.84-1.99)	1.07 (0.86-1.33)	0.85 (0.65-1.12)
p-value for interaction		0.60	0.71	0.60	0.64	0.71	0.64
Study site							
Arizona	1252	0.84 (0.44-1.63)	0.93 (0.50-1.73)	1.14 (0.68-1.93)	1.22 (0.67-2.20)	1.04 (0.76-1.42)	0.88 (0.61-1.29)
Oklahoma	1104	0.69 (0.34-1.40)	0.87 (0.43-1.78)	1.34 (0.77-2.35)	1.57 (0.84-2.93)	1.07 (0.75-1.54)	0.75 (0.51-1.12)
North/South Dekota	1215	0.75 (0.52-1.10)	0.90 (0.64-1.27)	1.25 (0.93-1.69)	1.41 (0.93-2.13)	1.05 (0.89-1.25)	0.81 (0.62-1.05)
p-value for interaction		0.87	0.83	0.87	0.83	0.83	0.83
Smoking							
Never	1144	0.69 (0.42-1.12)	0.70 (0.46-1.07)	1.35 (0.91-1.99)	1.27 (0.71-2.28)	1.20 (0.97-1.48)	0.86 (0.59-1.24)
Former	1204	0.87 (0.47-1.59)	1.00 (0.56-1.80)	1.12 (0.69-1.81)	1.26 (0.72-2.20)	1.00 (0.74-1.34)	0.87 (0.61-1.23)
Current	1223	0.83 (0.53-1.29)	0.97 (0.64-1.48)	1.16 (0.81-1.65)	1.31 (0.85-2.01)	1.01 (0.82-1.25)	0.84 (0.64-1.11)
p-value for interaction		0.79	0.61	0.79	0.72	0.61	0.72
DM							
No	1819	0.60 (0.41-0.87)	0.73 (0.51-1.04)	1.51 (1.12-2.03)	1.65 (1.12-2.42)	1.17 (0.98-1.41)	0.73 (0.57-0.93)
Yes	1752	0.94 (0.58-1.54)	1.06 (0.67-1.69)	1.05 (0.71-1.54)	1.16 (0.73-1.84)	0.97 (0.77-1.22)	0.91 (0.68-1.22)
p-value for interaction		0.12	0.16	0.12	0.24	0.16	0.24
Obesity							
BMI< 30 kg/m^2	1756	0.70 (0.50-0.97)	0.85 (0.63-1.15)	1.33 (1.02-1.73)	1.50 (1.04-2.15)	1.08 (0.93-1.26)	0.77 (0.62-0.97)

BMI≥ 30 kg/m^2	1815	0.97 (0.57-1.67)	1.02 (0.60-1.73)	1.02 (0.67-1.57)	1.06 (0.66-1.71)	0.99 (0.76-1.29)	0.96 (0.71-1.30)
p-value for interaction		0.15	0.19	0.15	0.28	0.19	0.28
Waist-hip ratio							
Non-abdominal obesity*	1002	0.68 (0.43-1.06)	0.75 (0.50-1.14)	1.36 (0.95-1.95)	1.39 (0.90-2.17)	1.15 (0.94-1.42)	0.81 (0.61-1.07)
Abdominal obesity	2569	0.87 (0.59-1.30)	0.99 (0.67-1.46)	1.11 (0.81-1.53)	1.22 (0.84-1.78)	1.01 (0.83-1.23)	0.88 (0.69-1.12)
p-value for interaction		0.18	0.12	0.18	0.48	0.12	0.48
iAs+MMA+DMA							
≤ 5.5 μg/g	897	0.78 (0.34-1.81)	0.81 (0.34-1.94)	1.22 (0.62-2.38)	1.21 (0.63-2.33)	1.11 (0.72-1.71)	0.89 (0.59-1.34)
> 5.5 & ≤ 8.8 μg/g	891	1.10 (0.52-2.35)	1.15 (0.53-2.51)	0.92 (0.51-1.69)	0.98 (0.52-1.87)	0.93 (0.63-1.38)	1.01 (0.67-1.51)
>8.8 & ≤ 13.9 μg/g	893	0.51 (0.32-0.81)	0.68 (0.46-0.99)	1.70 (1.18-2.46)	1.95 (1.13-3.38)	1.22 (1.01-1.47)	0.65 (0.46-0.93)
> 13.9 μg/g	890	0.90 (0.51-1.60)	1.03 (0.60-1.76)	1.08 (0.69-1.71)	1.21 (0.69-2.12)	0.99 (0.75-1.29)	0.89 (0.62-1.27)
p-value for interaction		0.40	0.51	0.40	0.58	0.50	0.58
Overall	3404	0.73 (0.55-0.98)	0.85 (0.65-1.12)	1.28 (1.02-1.62)	1.40 (1.04-1.87)	1.08 (0.95-1.24)	0.81 (0.67-0.97)

CHAPTER 3: Arsenic exposure, arsenic metabolism, and incident diabetes in the Strong Heart Study

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Index Words: arsenic, arsenic metabolism, arsenic methylation, diabetes

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Abstract

Objective

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

Research Design and Methods

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

Results

Over 11,263.2 person-years of follow-up, 396 participants developed diabetes. Using the leave-one-out approach to model the dynamics of arsenic metabolism, we found increasing MMA% was associated with decreased diabetes incidence. The hazard ratios (95% CI) of diabetes incidence for an IQR change in MMA% were 0.69 (0.52, 0.90) and 0.76 (0.65, 0.89) when iAs% and DMA% were, respectively, left-out of the model. DMA% was associated with increased diabetes incidence only when MMA% decreased (left-out from the model), but not when iAs% decreased. iAs% was also associated with increased diabetes incidence when MMA% decreased. The association between MMA%

and diabetes incidence was similar by age, sex, study site, obesity, and arsenic exposure status.

Conclusions

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

Background

Humans are exposed to inorganic arsenic through drinking water, food, dust, and ambient air.[1] Increasing epidemiologic and experimental evidence supports a role for inorganic arsenic in the development of diabetes mellitus.[2, 3] At high arsenic levels (>150 μg/L in drinking water), evidence from Taiwan and Bangladesh supports an association with diabetes, although most studies are cross-sectional and there are concerns about measures of arsenic exposure and the definition of diabetes in some studies.[2, 4] At low-moderate arsenic levels, recent evidence from Mexico and the United states, including cross-sectional[5, 6] and prospective studies[7, 8] support the role of arsenic in diabetes development.

Little is known, however, about the association between arsenic metabolism and diabetes. After absorption, inorganic arsenic (iAs; arsenate and arsenite) is methylated, primarily in the liver, to form monomethylated (MMA) and dimethylated (DMA) arsenic compounds, which are excreted into the urine together with iAs.[9, 10] Higher MMA% and lower DMA% in urine have been related to increased risk of cancer[11-13] and cardiovascular disease in studies from Taiwan and Bangladesh.[14, 15] The increased risk of cancer and cardiovascular disease associated with higher MMA% in urine may be related to the high toxicity of MMA (III).[16, 17] DMA is regarded as a less toxic arsenic species, as DMA is more rapidly excreted through the urine compared to inorganic arsenic.[18, 19] DMA (III), however, has been recently linked to the prevalence of diabetes in cross-sectional studies from Mexico and Bangladesh.[5, 20] Higher DMA% and lower MMA% has also been related to obesity in studies from Mexico and the US,[21, 22] although the temporality of these associations is unclear. In addition, arsenic metabolism is tightly

connected with one-carbon metabolism,[23] which has been implicated in both cancer development and cardiovascular disease,[24, 25] and may also play a role in diabetes.[26, 27] These findings highlight the need to properly evaluate the role of arsenic methylation profiles in diabetes development.

In this study, we investigated the associations of low-moderate arsenic exposure and arsenic metabolism with diabetes in the Strong Heart Study (SHS). The SHS is a population-based prospective cohort study of cardiometabolic diseases among 3 American Indian communities in rural Arizona, Oklahoma, and North and South Dakota ("the Dakotas").[28] In participants from Arizona and the Dakotas, drinking water was probably the major source of inorganic arsenic while in participants from Oklahoma, diet, including rice, flour and other grains, was probably the main source. Urine arsenic concentrations and measures of arsenic metabolism were stable in SHS participants during the time of follow-up, supporting the use of urine arsenic as a suitable surrogate for chronic arsenic exposure and metabolism.[29] In the SHS, we recently found that higher inorganic arsenic exposure was associated with higher diabetes prevalence,[6] supporting the need to further investigate the prospective associations between arsenic exposure and metabolism with diabetes incidence.

Method

Study population

In 1989-1991, the Strong Heart Study examined 4,549 American Indian men and women aged 45 to 74 years at baseline enrollment from 13 tribes and communities.[30] All

community members were invited to participate in Arizona and Oklahoma, whereas a cluster sampling procedure was used in the Dakotas.[31, 32] The overall participation rate was 62%. Compared with nonparticipants, participants were similar in age, body mass index, and prevalence of self-reported diabetes but were more likely to be female and to have self-reported hypertension.[32] Participants were invited to subsequent clinical visits between 1993 and 1995, and between 1998 and 1999.[31, 32] The SHS population is very stable, with low migration rates due to strong cultural and social links in the community.[33] The Indian Health Service, institutional review boards, and participating communities approved the study protocol. All participants provided informed consent.

The prevalence of diabetes in the Strong Heart Study in 1989-1991 was 50%. For this study, we used data from participants free of diabetes and with sufficient urine available for arsenic measurements at the baseline visit (N=1,986) (Supplementary figure 1). We further excluded 117 participants lost during follow up or missing both fasting glucose and 2-hour plasma glucose data during follow-up, 105 participants with inorganic or methylated arsenic species below the limit of detection as it is difficult to estimate arsenic methylation in these participants, and 70 participants missing other variables of interest leaving 1,694 participants for this analysis.

Data Collection

Baseline clinical information consisted of a personal interview, physical examination, fasting blood sample, and spot urine sample.[31] Sociodemographic (age, sex, and education) and lifestyle (smoking and alcohol status) information was collected by trained and certified interviewers using standardized questionnaires.[31] Physical examination measurements (height, weight, waist and hip circumferences, and systolic

and diastolic pressures) and bio-specimen collection (blood and urine) were conducted by centrally trained nurses and medical assistants following a standardized protocol.[31] Detailed procedures of clinical and laboratory examinations have been described.[31] Estimated glomerular filtration rate at baseline was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[34] Participants were asked to fast for 12 hours before blood samples were collected in the morning, at baseline and in the two subsequent visits. Spot urine samples were also collected in the morning and were frozen with 1 to 2 hours of collection. The biospecimens were stored at -70°C or lower before analyses.[31]

Diabetes measurements

Fasting plasma glucose level was determined by a hexokinase method at MedStar Health Research Institute, Washington, DC. A 2-hour, 75g oral glucose tolerance test was performed on all participants except those who were under insulin therapy, remained with poor glycemic control on oral medication, or had a fasting glucose level greater than 225 mg/dL determined by Accu-Chek II (Baxter Healthcare, Grand Prairie, Texas).[31] Glycated hemoglobin was measured at the laboratory of the National Institute of Diabetes and Digestive and Kidney Diseases Epidemiology and Clinical Research Branch, Phoenix, Arizona, by a high-performance liquid-chromatographic (HPLC) method. Diabetes was defined as a fasting plasma glucose ≥126 mg/dL, plasma glucose ≥200 mg/dL 2–h after ingestion of 75 g oral glucose load, self-reported diabetes history, and self-reported use of insulin or oral hypoglycemic medications.

Urine arsenic

To assess long-term arsenic exposure, we measured urine arsenic species after confirmation that concentrations were stable over a 10-year period.[29] Detailed analytic methods and associated quality control procedures for arsenic analysis have been published.[35] Arsenic species concentrations were determined by high-performance liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS) that served as the arsenic selective detector (Agilent 1100 HPLC and Agilent 7700x ICP-MS, Agilent Technologies, Santa Clara, California). Arsenic speciation can discriminate species directly related to iAs exposure (arsenite, arsenate, monomethylarsonate [MMA], and dimethylarsinate [DMA]) from those related to organic arsenicals in seafood (arsenobetaine as an overall marker of seafood arsenicals), which are generally considered nontoxic.[36] Urine concentrations of arsenobetaine and other arsenic cations were very low (median, 0.71; interquartile range, 0.41 to 1.69 μg/g creatinine), confirming that seafood intake was low in this sample, and indicating that DMA mainly came from inorganic arsenic exposure.[37] The limit of detection for total arsenic and for iAs (arsenite plus arsenate), MMA, DMA, and arsenobetaine plus other arsenic cations was 0.1 μg/L. Because a major goal of the study was to evaluate the role of arsenic metabolism in diabetes development, we excluded participants with iAs (5.2%), MMA (0.8%) and DMA (0.03%) below the limit of detection. An in-house reference urine and the Japanese National Institute for Environmental Studies No. 18 Human Urine were analyzed together with the samples. Interassay coefficients of variation for iAs, MMA, DMA and arsenobetaine for the in-house reference urine were 6.0%, 6.5%, 5.9%, and 6.5%, respectively.[35]

Statistical methods

We used the sum of urine inorganic (iAS; arsenite and arsenate) and methylated arsenic species (MMA and DMA) as the biomarker of inorganic arsenic exposure from multiple sources. We used the proportions of urine iAs, MMA and DMA over the sum of inorganic and methylated species, expressed as iAs%, MMA%, and DMA%, to evaluate arsenic metabolism. We graphically described the distribution of arsenic metabolism in people with and without diabetes using a triplot, a diagram with 3 axis that is well-suited to represent arsenic metabolism (Figure 1).

The prospective associations between arsenic exposure and arsenic metabolism with incident diabetes were evaluated by Cox proportional hazards models. Arsenic exposure was evaluated based on the urinary concentration of the sum of inorganic and methylated arsenic species. We also evaluated the urinary concentration of iAs, MMA and DMA in separate models. Arsenic metabolism was evaluated as iAs%, MMA% and DMA%. Similar to previous studies,[20, 38, 39] we first entered each arsenic metabolism biomarker alone in the regression model together with the sum of inorganic and methylated arsenic species to adjust for arsenic exposure. Entering each biomarker alone is difficult to interpret, as the increase in iAs, for instance, could be related to a decrease in either MMA or DMA. To address this problem, we used a "leave-one-out" approach. In this method, two biomarkers are entered at a time, e.g. iAs% and MMA%, leaving out the third one, DMA%, while holding constant urine arsenic concentrations. In the example, the regression coefficients for iAs% and MMA% estimate the hazard ratio associated with an increase in iAs% by decreasing DMA%, and with an increase in MMA% by decreasing DMA%, respectively. This method is used in the nutrition

literature to estimate the specific contribution of different macronutrients beyond their contribution to total energy intake as well as in the hematology literature to estimate the specific contribution of different blood cell types beyond total cell count.[40, 41]

All arsenic variables were modeled per interquartile range increment (in the log scale for urine arsenic species concentrations and in the original scale for % species) and using restricted cubic splines. We also modeled them using quartiles with similar findings (data not shown). The time scale for survival analysis was age. To handle left-truncation induced at time of enrollment and appropriately aligning risk sets on the age scale, the late entry method was conducted using age at baseline as the individual entry time. Models were adjusted progressively. Initially, we adjusted for sex and education (no high school, some high school, and high school or more). We then adjusted further for smoking and alcohol drinking status. Finally, we further adjusted for body mass index and waist–hip ratio. All models were adjusted for urine creatinine to account for urine dilution. In an alternative analysis we adjusted for specific gravity instead of urine creatinine. Both models yielded similar results (models for specific gravity are not shown). We confirmed that the proportional hazards assumption was fulfilled based on Schoenfeld residuals.

We conducted additional sensitivity analyses to evaluate the robustness of our primary findings. First, we evaluated the prospective associations of arsenic exposure and arsenic metabolism with incident diabetes by fitting generalized gamma distributions to survival times. Model selection was based on the Akaike Information Criterion (AIC) and estimates for the shape parameter indicated that log-normal distributions were appropriate. This approach yielded consistent findings as the Cox proportional hazards

model (data not shown). Second, because mortality rate was high in the SHS population,[42] we conducted competing risk analysis of death based on Fine and Gray's method, which yielded similar statistical inference.[43] We also used generalized gamma modeling to describe the competing relationship between mortality and incident diabetes according to arsenic exposure status (supplementary figure 2).[44] Third, we repeated the analysis for arsenic exposure including participants who had iAs, MMA or DMA below the limit of detection (LOD) by replacing levels below the LOD by the LOD divided by the square root of 2, also with similar findings (not shown).

All statistical analyses were performed in Stata/IC, version 12 (StataCorp, College Station, Texas) and R, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria [www.r-project.org]).

Results

The median urine concentration of inorganic plus methylated arsenic species was 10.2 μg/L (interquartile range, 6.1 to 17.7 μg/L). Urine arsenic concentrations were higher in participants from Arizona (median 14.3 μg/L), followed by the Dakotas (11.9 μg/L) and Oklahoma (median 7.0 μg/L). The median (interquartile range) for iAs%, MMA% and DMA% was 8.3 (5.7 to 11.3)%, 15.2 (11.7 to 18.8)% and 76.4 (70.3 to 81.4)%, respectively. Men, participants from the Dakotas, current smokers and participants with lower body mass index had higher MMA%, and correspondingly lower DMA% (Figure 2).

Over 11,263.2 person-years of follow-up, 396 participants developed diabetes. Diabetes incidence was 35.2 per 1000 person-years. Participants with incident diabetes were more likely to be female, from Arizona, and obese at baseline (Table 1). Younger age was borderline associated with incident diabetes (p-value 0.05). Urine concentrations of inorganic plus methylated arsenic were similar in participants with and without incident diabetes. Participants with incident diabetes had lower MMA% and higher DMA% compared to those without incident diabetes (Table 1, Figure 1). Arsenic exposure, assessed as the summed concentrations in urine of inorganic and methylated arsenic species or as each of the individual arsenic species, was not associated with incident diabetes in any of the multivariable adjusted models (Table 2 and Supplement Figure 3).

For arsenic metabolism, the multi-adjusted hazard ratio (95% CI) of diabetes incidence per IQR in arsenic metabolism biomarkers entered one-by-one in the model (conventional approach) was 1.00 (0.87-1.14) for iAs%, 0.79 (0.68-0.92) for MMA% and 1.17 (1.00-1.36) for DMA% (Table 3, model 4). Using the leave-one-out approach, we confirmed that increasing MMA% was associated with decreased diabetes incidence. The hazard ratios (95% CI) of diabetes incidence for an IQR change in MMA% were 0.69 (0.52, 0.90) and 0.76 (0.65, 0.89) when iAs% and DMA% were, respectively, left-out of the model (Table 3, model 4). Consistently, increasing MMA% was related to decreased diabetes incidence in flexible dose-response analyses when either iAs% or DMA% were left-out of the model (Figure 3). DMA% was associated with increased diabetes incidence only when substituted for MMA% and iAs% was associated with increased diabetes incidence only when substituted for MMA% (Table 3, Figure 3).

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

Discussion

Arsenic metabolism, but not inorganic arsenic exposure, was prospectively associated with diabetes incidence in American Indians from Arizona, Oklahoma and North/South Dakota. Higher iAs% and DMA% in urine, because of lower MMA%, was associated with higher diabetes incidence. Consistently, higher MMA% was associated with lower risk of diabetes. The associations persisted after adjustment for sociodemographic factors, smoking, alcohol, kidney function, and measures of adiposity. These novel findings support that arsenic metabolism patterns, in particular lower MMA%, may be a predisposing factor for diabetes. Arsenic exposure, measured by the concentration of inorganic plus methylated arsenic species in urine, however, was not associated with diabetes incidence in our study population. The study was conducted in a population with a high burden of obesity and diabetes[45] and characterized by low-to-moderate arsenic exposure levels.

Non-genetic determinants of arsenic metabolism include sex (women have higher DMA% than men), smoking (never smokers generally have higher DMA% than current smokers), nutritional status (dietary folate and vitamin deficiencies are associated with lower DMA%), and BMI (obese participants have higher DMA%).[9] In women, MMA% decreases and DMA% increases during pregnancy.[46, 47] While the risk of gestational

diabetes is also increased, a connection with changes in arsenic metabolic patterns during pregnancy is unknown.[48, 49] Interestingly, in our study the arsenic metabolic pattern associated with increased diabetes risk paralleled that observed during pregnancy, i.e., lower MMA% and higher DMA%. Genetic determinants, especially variants in arsenic (III) methyltransferase (*AS3MT*), have also been related with arsenic methylation patterns in urine.[50, 51] Additional research is needed to evaluate whether genetic variants play a role in the connection between arsenic metabolism profile and diabetes.

Little is known about arsenic metabolism and diabetes as compared to its role in cancer and cardiovascular disease.[14, 52-55] In those studies, conducted mostly in Taiwan and Bangladesh, higher MMA% was associated with the development of lung[54], bladder[52] and skin[53] cancers and with cardiovascular disease including atherosclerosis and peripheral vascular disease.[14, 55] In one small case-control study from Bangladesh, higher DMA% was associated with increased prevalence of diabetes, although the association was not statistically significant.[20] High BMI has also been significantly associated with low MMA% and high DMA% in urine in adults from Mexico and the Strong Heart Study.[21, 22] In our study, adjusting for baseline BMI and waist-hip ratio slightly attenuated the association between arsenic metabolism and incident diabetes, although the association remained. How this specific pattern (low MMA% with either high iAs% or DMA%) may affect individual susceptibility to endocrine and metabolic diseases remains unclear.

Substantial experimental research supports the role of arsenic exposure in diabetes development.[2, 3] Experimental studies, in general, have not focused on differences by arsenic metabolism. High MMA% may be considered as a marker of insufficient

methylation capacity to DMA. Recent experimental studies have shown that methylation could be a bio-activation process, with DMA(III) being a potent and highly toxic dimethylated arsenic species.[56, 57] DMA(III) was found to impair insulin signaling and glucose homeostasis.[17, 58] In a cross-sectional study from Mexico, the concentrations of DMA(III) in urine were associated with diabetes.[5] In our study, similar to other large epidemiologic studies, we measured total MMA and DMA, as MMA(III) and DMA(III) are unstable in urine and quickly revert to their pentavalent forms.[59]

The association of arsenic metabolism with diabetes could also be related to one carbon metabolism, as S-adenosylmethionine (SAM) is the methyl donor for arsenic metabolism.[24, 60] Recent experimental evidence has shown that SAM plays an important role in lipogenesis and in the development of diabetes.[26, 61, 62] An *in vitro* study in *Caenorhabditis elegans*, an experimental model for human diseases and metabolic pathways,[63, 64] found that the synthesis of SAM regulated the expression of genes required for adequate lipid metabolism.[61] In *HepG2* human hepatocytes, the optimal balance between SAM and S-adenosylhomocystine (SAH) was critical to maintain appropriate expression of gluconeogenic enzymes.[62] In addition, in a cross-sectional study of 50-75 year old adults from the Netherlands (N=648), plasma SAM was positively associated with fat mass and truncal adiposity, although reverse causation could not be excluded.[65] We cannot discount the possibility that arsenic metabolism acts as a marker of one carbon metabolism. In any case, our findings indicate that more research is warranted to understand the impact of arsenic methylation and other methylation processes related to one-carbon metabolism on the development of diabetes.

In our study, we found no association between arsenic exposure and incident diabetes, although cross-sectionally we had found an association.[6] Inorganic arsenic and its methylated metabolites may induce diabetes by impairing insulin production by pancreatic ß cells or inhibiting basal or insulin-stimulated glucose uptake by peripheral tissues.[10, 66] Relevant mechanisms by which arsenic could affect ß-cell function and insulin sensitivity include oxidative stress, glucose uptake and transport, gluconeogenesis, adipocyte differentiation, calcium signaling, and epigenetic effects.[2, 10] A number of recent studies have reported a prospective association between arsenic exposure and diabetes development [3, 7, 8] It is possible that arsenic exposure is not a risk factor for diabetes in our population. At the same time, the presence of an association between arsenic exposure and diabetes cross-sectionally but not prospectively could be related to competing risk of premature death and differential survival bias that may mask the true association in our population. Because arsenic was strongly associated with diabetes at baseline and the prevalence of diabetes at baseline was 50%,[6] another possible explanation for the lack of association is that the pool of susceptible participants is too small for the association to be seen prospectively. In support of this possibility, age was not positively associated with diabetes incidence either (Table 1). BMI, however, remained a strong risk factor.

Strengths of our study include standardized protocol to collect data over the follow-up, high-quality laboratory methods for measuring concentrations of urine arsenic species at baseline and careful modeling of the dynamic of arsenic metabolism including the leave-one-out approach. This study had several limitations. First, the urine arsenic concentrations and metabolism were measured in a single sample at baseline to represent

internal doses and individual metabolism profiles. However, we have confirmed that arsenic levels in urine and arsenic metabolism were constant over 10-years in this population.[29] Second, adjustment for adiposity could induce over-adjustment as obesity may be in the causal pathway between arsenic metabolism and diabetes. Finally, our population was between 40 and 74 years of age and the burden of diabetes at baseline was already 50%. It is thus possible that participants susceptible of developing diabetes at baseline were different from the source population. Studies in younger populations with a lower prevalence of diabetes at baseline are needed.

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

Table 1. Characteristics of Strong Heart Study participants free of diabetes at baseline (1989-1991).

	No DM event n=1,298, 76.6%		**DM events n=396, 23.4%**		***p*-value**
	N (%)	**Median(IQR)**	**N %**	**Median(IQR)**	
Age, year		54.6 (48.8-61.8)		53.3 (48.5-60.3)	0.05
Male	610 (47.0)		153 (38.6)		<0.01
Location					
Arizona	255 (19.7)		114 (28.8)		<0.01
Oklahoma	504 (38.8)		109 (27.5)		
North and South Dakota	539 (41.5)		173 (43.7)		
Education (yrs)					0.06
No high school	230 (17.7)		91 (23.0)		
Some high school	305 (23.5)		89 (22.5)		
High school or more	763 (58.8)		216 (54.6)		
Smoking (%)					0.05
Never	353 (27.2)		126 (31.8)		
Former	398 (30.7)		129 (32.6)		
Current	547 (42.1)		141 (35.6)		
Alcohol (%)					0.19
Never	158 (12.2)		61 (15.4)		
Former	499 (38.4)		154 (38.9)		
Current	641 (49.4)		181 (45.7)		
Body mass index		28.0 (25.0-31.9)		30.9 (28.1-35.3)	<0.01
Waist-hip ratio		0.94 (0.89-0.98)		0.96 (0.92-0.99)	<0.01
Waist circumference (cm)		98 (91-107)		106 (98-116)	<0.01
% Body fat		33.3 (27.1-40.8)		38.5 (31.1-44.3)	<0.01
Urine creatinine, g/L		1.3 (0.8-1.8)		1.2 (0.9-1.7)	0.80
eGFR , ml/min/1.73m^2		81.3 (71.6-92.7)		81.1 (70.8-93.7)	0.48

Fasting glucose, mg/dL		100 (93-107)		106 (98-113)	<0.01
HbA1c, %	N=1,214	5.0 (4.7-5.4)	N=375	5.3 (4.9-5.7)	<0.01
Arsenic exposure					
iAs + methylated arsenic*, μg/g		8.7 (5.3-13.8)		9.1 (5.9-14.0)	0.32
iAs, μg/g		0.7 (0.4-1.4)		0.7 (0.4-1.3)	0.87
MMA, μg/g		1.3 (0.8-2.2)		1.2 (0.8-2.1)	0.58
DMA, μg/g		6.4 (4.0-10.3)		7.0 (4.4-11.2)	0.16
Arsenic metabolism					
iAs%		8.4 (5.7-11.6)		8.1 (5.7-10.7)	0.09
MMA%		15.5 (12.0-19.1)		14.0 (11.2-17.1)	<0.01
DMA%		75.9 (69.6-81.3)		77.4 (72.6-81.9)	<0.01

Abbreviations: iAs, inorganic arsenic including arsenate and arsenite; DM, diabetes mellitus; DMA, dimethylarsinate; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c, MMA, methylarsonate;

Table 2. Hazard ratios (95% confidence intervals) for incident diabetes per interquartile range in urine concentrations of inorganic arsenic (iAs), monomethylarsonate (MMA), dimethylarsinate (DMA) and the sum of iAs, MMA and DMA (μg/g creatinine).

Arsenic (interquartile range)	Model 1	Model 2	Model 3	Model 4
iAs (0.4-1.4 μg/g)	0.87 (0.74-1.01)	0.86 (0.74-1.01)	0.88 (0.75-1.04)	0.95 (0.81-1.12)
MMA (0.8-2.2 μg/g)	0.78 (0.66-0.91)	0.76 (0.64-0.89)	0.77 (0.65-0.90)	0.85 (0.72-1.00)
DMA (4.0-10.5 μg/g)	1.00 (0.86-1.17)	0.95 (0.81-1.11)	0.96 (0.82-1.13)	0.98 (0.83-1.15)
iAs + methylated arsenic (5.5-13.9 μg/g)	0.94 (0.81-1.09)	0.89 (0.76-1.05)	0.91 (0.77-1.07)	0.95 (0.81-1.11)

Model 1: Stratified by study center and adjusted for age (age as time metric and age at baseline were treated as staggered entries)
Model 2: Further adjusted for sex, education
Model 3: Further adjusted for smoking, alcohol drinking
Model 4: Further adjusted for body mass index and waist-hip ratio

Table 3. Hazard ratios (95% confidence intervals) for incident diabetes per interquartile range in arsenic metabolism biomarkers (inorganic arsenic% [iAs%], monomethylarsonate% [MMA%] and dimethylarsinate [DMA%]). As the three biomarkers equal 100%, models entered two biomarkers at a time. All models adjusted for the sum of iAs, MMA and DMA (µg/g creatinine).

Arsenic metabolism (interquartile range)	Model 1	Model 2	Model 3	Model 4
Conventional approach				
iAs% (5.7-11.3)	0.88 (0.78-1.00)	0.91 (0.80-1.04)	0.92 (0.81-1.05)	1.00 (0.87-1.14)
MMA% (11.7-18.8)	0.69 (0.60-0.80)	0.70 (0.60-0.81)	0.70 (0.61-0.82)	0.79 (0.68-0.92)
DMA% (70.3-81.4)	1.35 (1.17-1.55)	1.33 (1.15-1.53)	1.31(1.13-1.52)	1.17 (1.00-1.36)
Leave-one-out approach				
↑iAs% corresponds to:				
↓MMA% (11.7-18.8)	1.37 (1.11-1.69)	1.39 (1.13-1.70)	1.40 (1.14-1.72)	1.35 (1.09-1.67)
↓DMA% (70.3-81.4)	1.01 (0.89-1.15)	1.03 (0.91-1.17)	1.04 (0.92-1.19)	1.09 (0.95-1.24)
↑MMA% corresponds to:				
↓iAs% (5.7-11.3)	0.67 (0.52-0.88)	0.66 (0.51-0.86)	0.66 (0.50-0.85)	0.69 (0.52-0.90)
↓DMA% (70.3-81.4)	0.69 (0.59-0.80)	0.69 (0.59-0.81)	0.69 (0.59-0.81)	0.76 (0.65-0.89)
↑DMA% corresponds to:				
↓iAs% (5.7-11.3)	0.97 (0.76-1.25)	0.94 (0.73-1.21)	0.92 (0.71-1.19)	0.85 (0.65-1.11)
↓MMA% (11.7-18.8)	1.80 (1.41-2.31)	1.79 (1.40-2.29)	1.79 (1.39-2.29)	1.54 (1.19-1.98)

In the conventional approach, each arsenic metabolism biomarker (iAs%, MMA%, and DMA%) is entered alone in the model. In the leave-one-out approach, two arsenic metabolism biomarkers are entered in the model. In that model, an increase in each arsenic metabolism biomarker corresponds to a decrease in the biomarker that is left out of the model. For instance, an increase in iAs% corresponds to a decrease in MMA% when we include DMA% in the model and leave MMA% out. The magnitude of the association for iAs% when MMA% is left-out will be the same but in opposite direction as for MMA% when iAs% is left-out. Both in the conventional approach and in the leave-one-out approach we adjusted for the sum of inorganic and methylated arsenic concentrations in urine to hold arsenic exposure levels constant.

Model 1: Stratified by study center, adjusted for age (age as time metric and age at baseline were treated as staggered entries) and adjusted for the sum of inorganic arsenic and methylated arsenic concentrations.

Model 2: Further adjusted for sex and education.

Model 3: Further adjusted for smoking and alcohol drinking status.

Model 4: Further adjusted for body mass index and waist-hip ratio.

Figure 1. The triplot presents the distribution of arsenic metabolism biomarkers in participants with and without incident diabetes (red dots and grey dots, respectively). The large red and grey solid dots represent the compositional arsenic metabolism mean for participants with and without incident diabetes, respectively. Inorganic arsenic (iAs) % is presented along the blue axis, monomethylarsonate (MMA) % along the red axis and dimethylarsinate (DMA) % along the green axis.

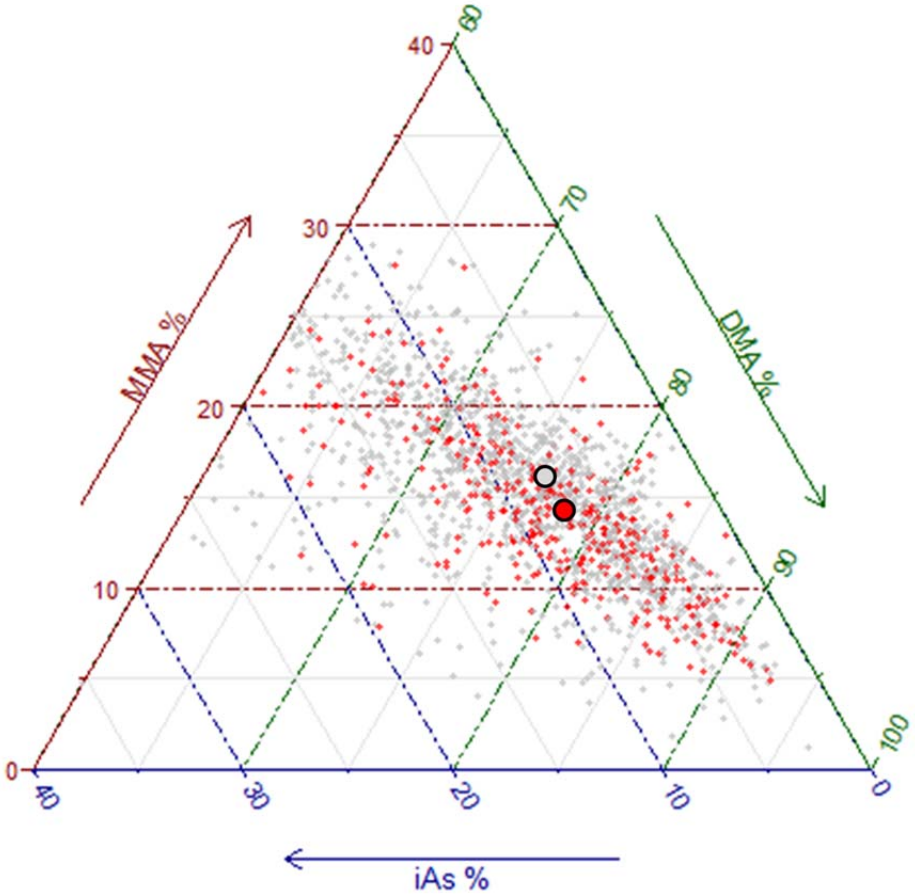


Figure 2. Median (IQR) of arsenic metabolism biomarkers by participant characteristics. The squares reflect the median for each arsenic metabolism biomarker and is proportional to sample size. The horizontal lines represent the interquartile range. The dashed vertical lines represent the overall median.

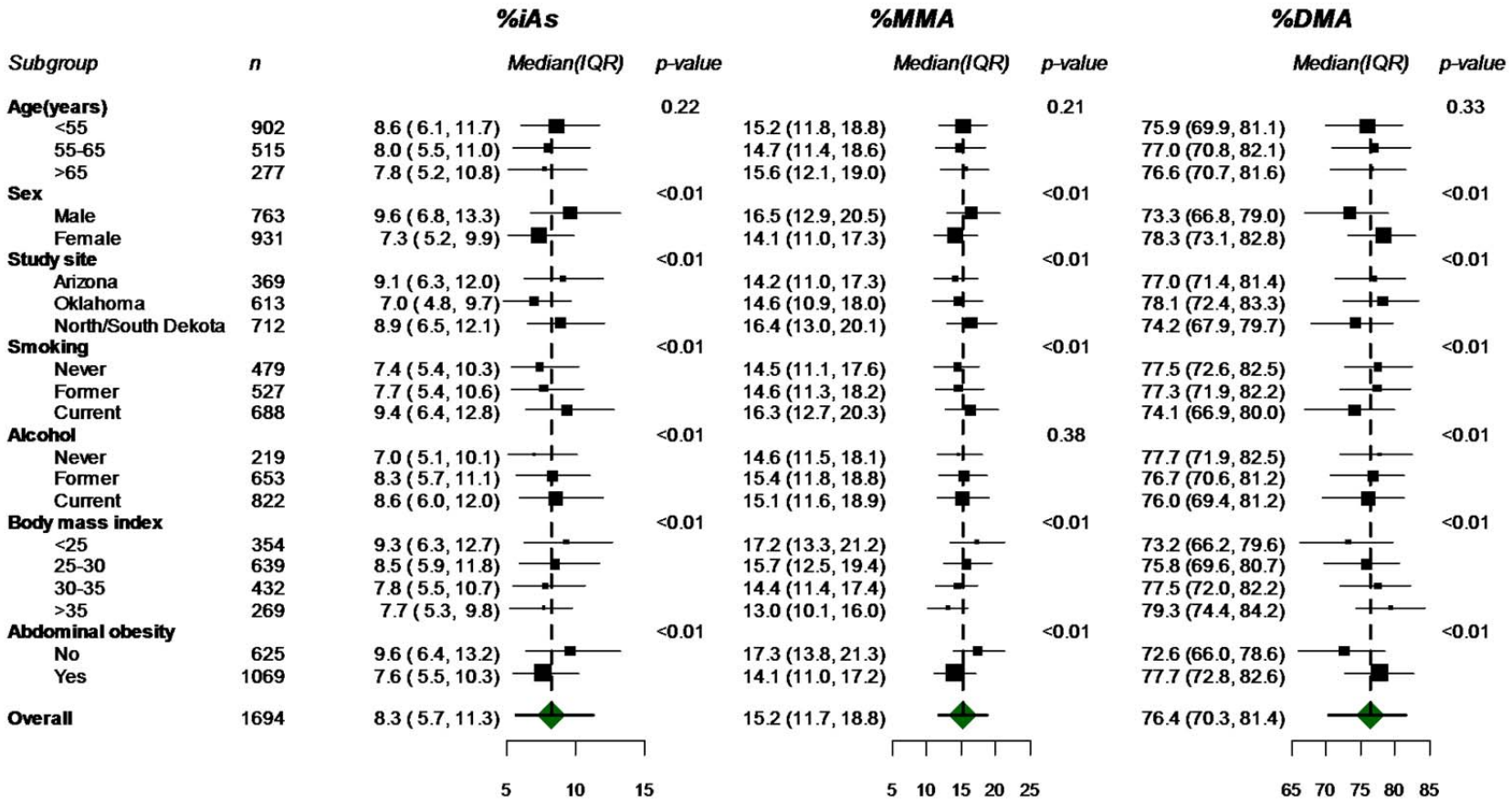
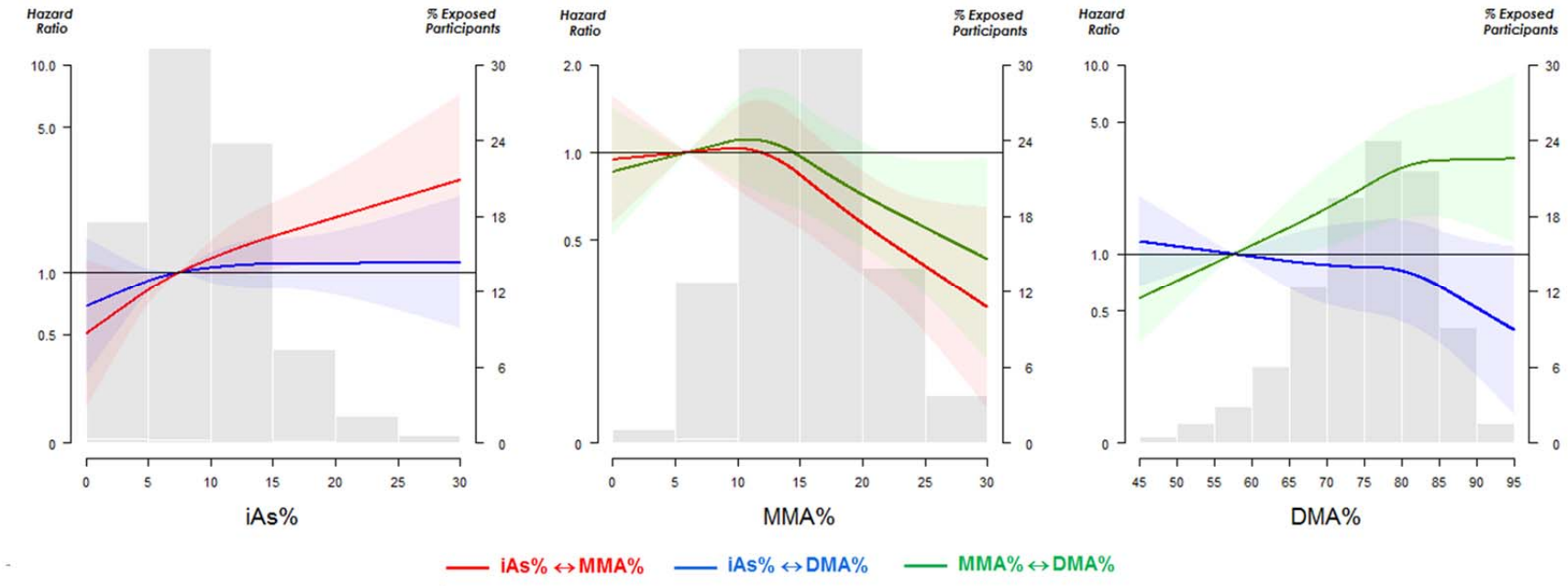


Figure 3. Hazard ratios for incident diabetes by arsenic metabolism biomarkers. Solid lines (shaded area) represent adjusted hazard ratios (95% confidence intervals) based on restricted quadratic splines with knots at the 10th, 50th, and 90th percentiles. The reference value was set at 10th percentile of each arsenic metabolism biomarker. The solid line represents the hazard ratio for iAs% when it replaces MMA% (red line) and DMA% (blue line) in the left panel, the hazard ratio for MMA% when it replaces iAs% (red line) and DMA% (green line) in the middle panel, and the hazard ratio for DMA% when it replaces iAs% (blue line) and MMA% (green line) in the right panel.



Supplementary table 1. Adjusted hazard ratios and 95% confidence intervals for the diabetes incidence comparing the 75th with 25th percentile of the sum of iAs, MMA and DMA (μg/g creatinine) and the % of monomethylarsonate [MMA%], by participant characteristics at baseline.

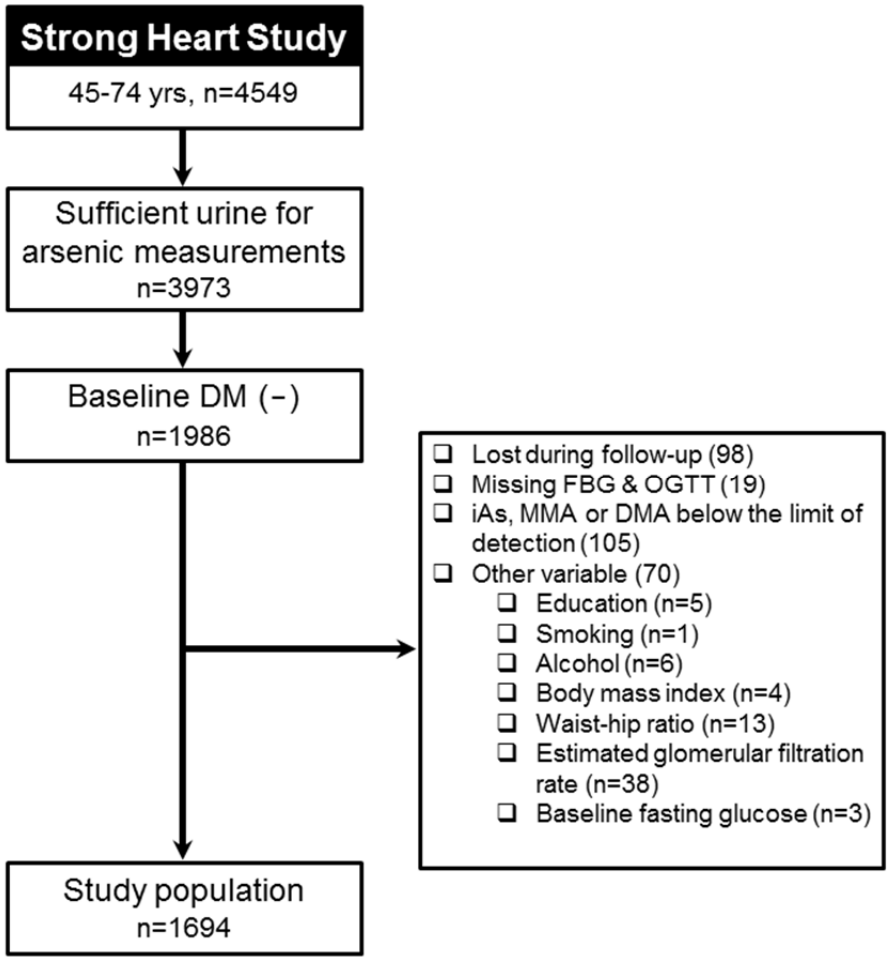
Subgroup	n	iAs+MMA+DMA (μg/g creatinine)	*P* value for interaction	MMA%**	*P* value for interaction
Age (years)					
< 55	902	0.88 (0.72-1.08)	0.47	0.80 (0.65-1.00)	0.31
≥ 55	792	1.00 (0.77-1.28)		0.67 (0.52-0.87)	
Sex					
Men	763	1.12 (0.87-1.43)	0.36	0.84 (0.66-1.07)	0.72
Women	931	0.86 (0.70-1.06)		0.69 (0.55-0.87)	
Study site					
Arizona	369	0.96 (0.68-1.34)	0.91	0.68 (0.48-0.96)	0.52
Oklahoma	613	0.94 (0.68-1.29)		0.69 (0.50-0.94)	
North/South Dekota	712	0.95 (0.76-1.18)		0.85 (0.67-1.07)	
Smoking					
Never	479	0.77 (0.57-1.03)	0.31	0.81 (0.60-1.11)	0.72
Former	527	1.14 (0.85-1.53)		0.68 (0.50-0.91)	
Current	688	1.03 (0.80-1.33)		0.83 (0.63-1.09)	
Obesity					
Non-obese (BMI< 30 kg/m^2)	993	0.84 (0.65-1.07)	0.39	0.73 (0.58-0.93)	0.62
Obese (BMI≥ 30)	701	1.02 (0.82-1.26)		0.76 (0.61-0.95)	
Waist-hip ratio					

Non-abdominal obesity*	625	1.01 (0.70-1.45)	0.75	0.64 (0.45-0.91)	0.23
Abdominal obesity	1069	0.94 (0.79-1.13)		0.79 (0.66-0.96)	
iAs+MMA+DMA					
≤ 5.5 μg/g creatinine	426	--		0.89 (0.63-1.28)	0.80
> 5.5 & ≤ 8.8 μg/g creatinine	421	--		0.63 (0.45-0.89)	
>8.8 & ≤ 13.9 μg/g creatinine	424	--		0.73 (0.52-1.02)	
> 13.9 μg/g creatinine	423	--		0.74 (0.53-1.03)	
Overall	1694	0.94 (0.80-1.11)		0.76 (0.65-0.89)	

*Abdominal obesity is defined as waist circumference>102 cm and >88 cm for men and women, respectively.

**In this model we adjusted for inorganic arsenic % and left out dimethylarsinate % (i.e. an increase in MMA% corresponds to a decrease in DMA%)

Supplementary figure 1. Flow chart of participant selection.



Abbreviations: iAs, inorganic arsenic; DMA, dymethylarsinate; FBG, fasting blood glucose; MMA, monomethylarsonate; OGTT, oral glucose tolerance test.

Supplementary figure 2. The mixture of generalized gamma distributions summarized the cumulative incidence of both diabetes and death in participants with highest and lowest quartile of arsenic exposure.

Brief Methods

The main competing event in our study was death. A total of 97 women and 128 men without developing diabetes died between 1989-1991 and December 31, 1999. Uncensored observations correspond to either the time when a participant developed diabetes or to the time when a participant died during follow-up. If π is the proportion of the total population of participants who developed diabetes and 1– π is the complementary proportion of patients who died without developing diabetes, we used a mixture according to π and 1- π of two generalized gamma distributions to model time to diabetes development and time to death.[1] We used the 3-parameter generalized gamma distribution (β for location, σ for scale, and **κ** for shape) for this analysis because of its flexibility to accommodate various hazard patterns. We modeled time to diabetes development with a generalized gamma distribution with density *f(t)*, and the times to death with another generalized gamma distribution with density *g(t)*. Hence, if T denotes the time to either diabetes development or death, the proportion with T < t is given by:

$$\Pr(T < t) = \Pr(T < t, event = diabetes) + Pr\ (T < t, event = death) = \ \pi\ [1 - F(t)] + (1 - \pi)\ [1 - G(t)]$$

where F and G were the survival functions corresponding to the *f* and *g* densities, respectively. $\pi\ [1 - F(t)]$ is the cumulative incidence of diabetes and $(1 - \pi)\ [1 - G(t)]$ is the cumulative incidence of death. The detailed analysis procedure has been described.[2] Publicly available algorithms at the Johns Hopkins STATPEI website (www.statepi.jhsph.edu) facilitated the development of the maximal likelihood function to fit these types of mixture models.

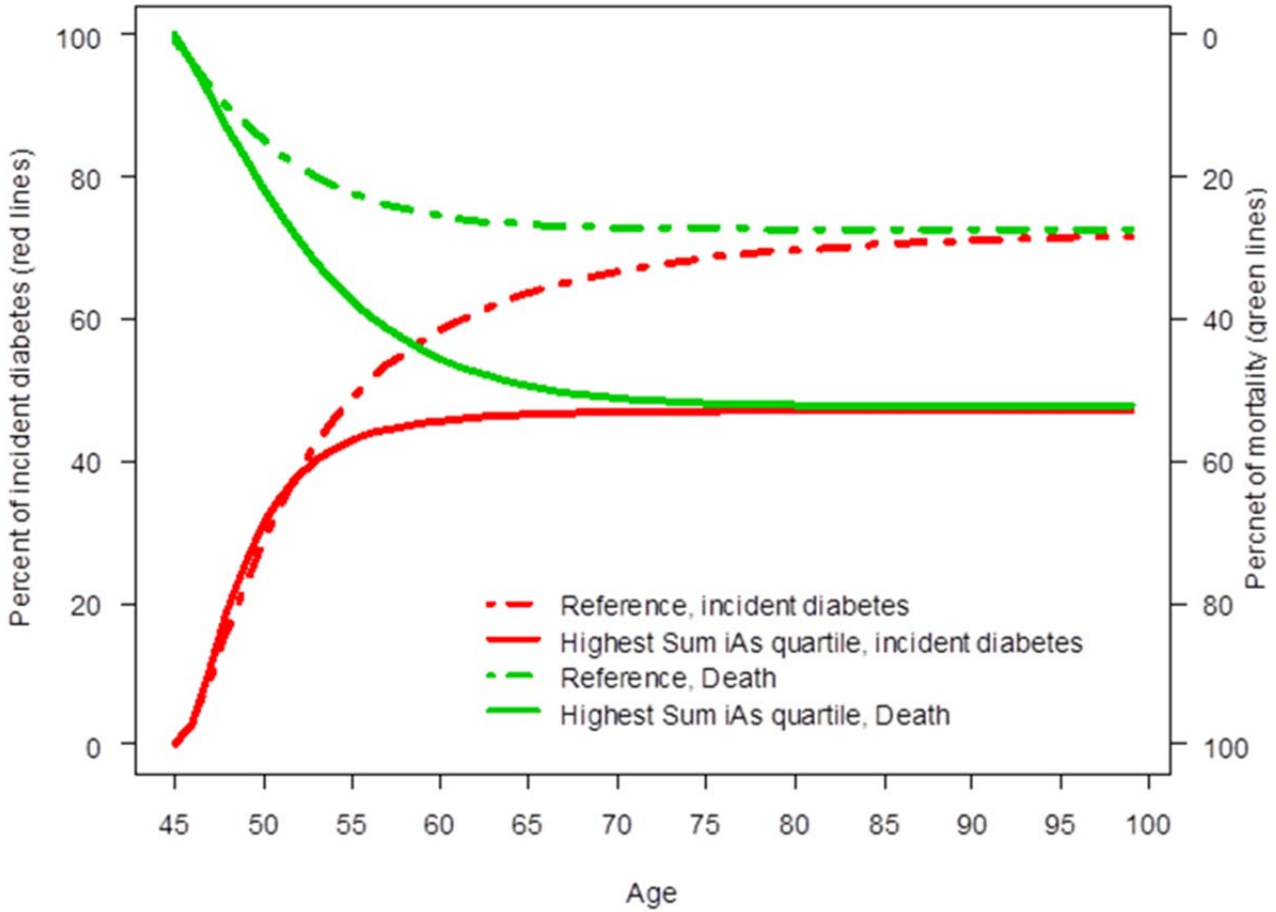
Competing risk graph using generalized gamma model – Sum of iAs and methylated As

Full Saturated Model		**Incident diabetes**			**Death**			
	%	β_1	σ_1	κ_1	β_2	σ_2	κ_2	AIC
Reference group (Quartile 1)	0.727	2.60	0.95	0.0001	2.56	0.87	1	5506.978
Arsenic (Quartile 2)	0.645	2.36	0.92	0.0001	2.65	0.60	1	
Arsenic (Quartile 3)	0.809	2.55	1.00	0.0001	1.90	0.64	1	
Arsenic (Quartile 4)	0.471	2.05	0.72	0.0001	2.83	0.80	1	

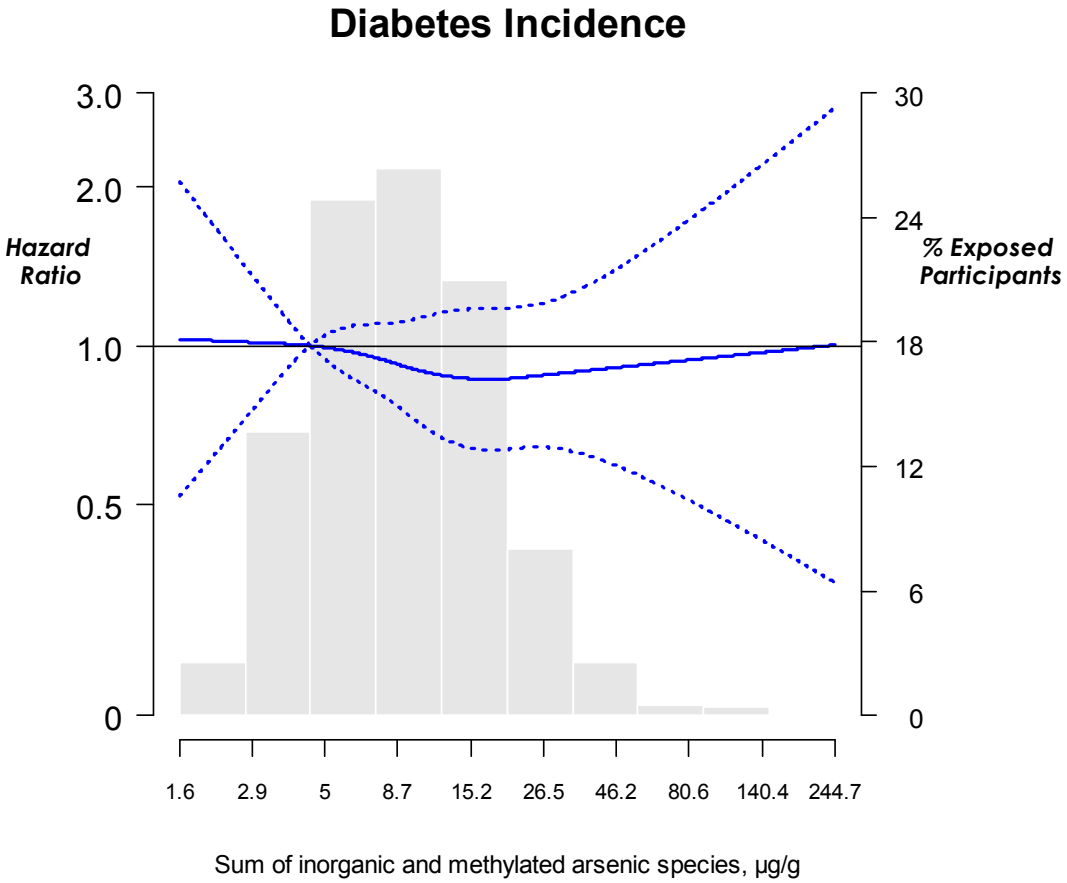
Reference:

1. Cox C, Chu H, Schneider MF, Munoz A. Parametric survival analysis and taxonomy of hazard functions for the generalized gamma distribution. *Statistics in medicine*. 2007;26:4352-4374

2. Checkley W, Brower RG, Munoz A, Investigators NIHARDSN. Inference for mutually exclusive competing events through a mixture of generalized gamma distributions. *Epidemiology*. 2010;21:557-565



Supplementary figure 3. Hazard ratios for incident diabetes by urine arsenic concentrations. Solid lines represent adjusted hazard ratios based on restricted quadratic splines for the log-transformed sum of inorganic and methylated arsenic species, with knots at the 10th, 50th, and 90th percentiles (3.8, 8.8, and 21.7 g/g creatinine, respectively). The dotted lines represent upper and lower 95% CIs. The reference was set at the 10th percentile of the arsenic distribution (3.8g/g creatinine).



CHAPTER 4: Arsenic metabolism and chronic kidney disease in the Strong Heart Study

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Abstract

Context: The role of arsenic metabolism in kidney disease remains unclear and relevant evidence is scarce.

Objective: To assess the prospective association between arsenic metabolism and kidney disease in American Indian population exposed to low-moderate levels of arsenic.

Design, Setting, and Participants: Prospective cohort study in 3,143 American Indian participants aged 45 to 75 years living in Arizona, Oklahoma, and North and South Dakota. The sum of urine inorganic arsenic (arsenite and arsenate), monomethylated (MMA), and dimethylated (DMA) arsenic compounds at baseline was used as the biomarker of inorganic arsenic exposure from multiple sources. The proportions of urine inorganic arsenic (arsenite and arsenate, iAs), MMA and DMA over the sum of inorganic and methylated species, expressed as iAs%, MMA%, and DMA%, was used to evaluate arsenic metabolism.

Main outcome measures: Kidney disease was determined by estimated glomerular filtration rate (eGFR), eGFR drop greater than 25%, macroalbuminuria, and renal replacement therapy requirements.

Results: The median (interquartile range) for inorganic arsenic%, MMA% and DMA% was 8.3 (5.8 to 11.2), 13.9 (10.8 to 17.4) and 77.5 (71.7 to 82.5), respectively. In multi-adjusted Cox proportional hazards model, the hazard ratio of incident kidney disease defined by reduced eGFR for an interquartile range increase in iAs% was 0.62 (95% CI 0.49-0.78) and 0.73 (0.63-0.85) when it substituted MMA% and DMA%, respectively. The adjusted hazard ratio was 1.23 (1.06-1.43) for an interquartile increase in MMA% with a corresponding decrease in DMA%. The results were robust to different kidney

disease definitions. The effects of replacing iAs% by MMA% or DMA% on the risk of developing kidney disease displayed a linear dose-response relationship and were enhanced among obese participants defined by body mass index higher than 30 kg/m^2.

Conclusion: Arsenic metabolism is independently and prospectively associated with the development of chronic kidney disease. Integrating the information of arsenic methylation capacity is therefore important for arsenic risk assessment. More research is needed to verify our results and further explore the pathogenesis of arsenic-related kidney injury from the viewpoints of systemic metabolism, epigenomics, and the interaction with other potential environmental nephrotoxicants.

Background

Arsenic (As) is a naturally-occurring element found in rock, soil and water and extensively used in anthropogenic activities leading to its ubiquitous presence in the environment. In addition to the well-recognized adverse effects of inorganic arsenic including carcinogenicity, cardiovascular toxicity, and diabetogenic potential, recent research has found the association between inorganic arsenic and chronic kidney disease (CKD).[1, 2] Although the molecular mechanisms underlying the pleiotropic effects of long-term arsenic exposure remains poorly understood, arsenic metabolism is considered to play a critical role as the elimination process of arsenic involving an interplay between arsenic methylation and one-carbon metabolism, a complex metabolic network linking to diverse diseases such as cancer and cardiovascular diseases.[3] In humans, absorbed inorganic arsenic (arsenate and arsenite) is primarily methylated in the liver to form monomethylated (MMA) and dimethylated (DMA) arsenic compounds, which are excreted into the urine with unchanged inorganic arsenic. The proportion of arsenic species in urine is 10-30% for inorganic arsenic, 10-20% for MMA, and 60-80% for DMA with substantial inter-individual variation.[4, 5] Increasing evidence has supported the different arsenic methylation patterns may associate with certain diseases and physiologic status. Higher MMA% in urine has been related to increased risk of cancer and cardiovascular disease while higher DMA% has also been related to diabetes and obesity.[1, 6-8] However, little is known about the association between arsenic metabolism and CKD.

CKD is a key determinant of poor health outcomes and imposes significant financial costs on health care system.[9, 10] The prevalence of CKD defined by reduced

estimated filtration rate (eGFR< 60 ml/min/1.73m^2) or albuminuria (urine albumin-to-creatinine ratio ≥ 30 mg/g) is 10% to 15% of adults in developed countries such as United States[11], Europe[12], and Asia[13] and may be even higher in developing countries including China[14] and the Central America.[15] The US Surgeon General's latest report, Healthy People 2020, has recognized CKD as an important public health issue and recommended systemic preventive strategies including early detection and effective treatment of CKD.[16] The first three leading causes of CKD in the United States include diabetes, hypertension, and glomerulonephritis.[17] However, the epidemiology of CKD is not consistent across countries. For instance, less than 20% of incident patients with end stage renal disease (ESRD) in Norway, Netherlands, and Russia are related to diabetes in contrast to about 45% and 60% in United States and Singapore, respectively.[17] The disproportionally high prevalence of diabetes among ESRD patients in certain countries supports the hypothesis that individual susceptibility to CKD may be intertwined with genetic and environmental factors such as pollution, nutritional or lifestyle transition, population growth and urbanization, or alteration of population age structure.[18]

This study examined the association between arsenic exposure and arsenic metabolism with incident CKD in the Strong Heart Study (SHS). Understanding the role of arsenic metabolism in the development of CKD is important to approach risk assessment from a differential-susceptibility perspective. The connection between arsenic metabolism and one-carbon metabolism may offer an opportunity for prevention strategies to ameliorate arsenic toxicity and modify individual susceptibility to CKD.

Methods

Study population

The Strong Heart Study (SHS) is a population-based study examining cardiovascular diseases and diabetes in men and women aged 45 to 74 years between 1989 and 1991 in 13 American Indian tribes and communities from Arizona, Oklahoma, and North and South Dakota.[19] All eligible community members were invited to participate in Arizona and Oklahoma, whereas a cluster sampling technique was applied in the Dakotas.[19] The overall participation rate was 62%, and a total of 4,549 participants were enrolled. Participants were then invited to subsequent clinical visits between 1993 and 1995 and between 1998 and 1999. The SHS was a stable population with low migration rate over the follow-up period as most adults live in the community of their birth and have strong cultural and social links in the community.[20, 21] Compared to nonparticipants, participants were similar in age, body mass index, and self-reported diabetes but were more likely to be female and to have self-reported hypertension.[22] The Indian Health Service, institutional review boards, and participating tribes approved the study protocol. All participants provided informed consent.

We used data from 3,973 participants with sufficient urine available for arsenic quantification at the baseline visit. We further excluded 228 participants with some arsenic species data (inorganic arsenic, MMA or DMA) below the limit of detection, 5 missing education, 10 missing smoking or alcohol drinking status, 42 missing body mass index or waist-hip ratio, 67 missing baseline estimated glomerular filtration rate (eGFR) or urine albumin-creatinine ratio (UACR), and 151 missing both eGFR measures during

follow-up, leaving 3,470 eligible participants. . We then excluded 327 participants with reduced eGFR < 60 ml/min/1.73m^2 or end stage renal disease (ESRD) on renal replacement therapy at baseline, leaving 3,143 participants in this analysis. Included participants were similar to those who were excluded because of missing data (data not shown).

Data collection

Baseline clinical information included a personal interview, physical examination, fasting blood test, and spot urine sample collection.[19] Socidemographic (age, sex, and education) and lifestyle (smoking and alcohol status) information was collected by trained and certified interviewers using standardized questionnaires.[19] Physical examination measurements (height, weight, waist and hip circumferences, and systolic and diastolic pressures) and bio-specimen collection (blood and urine) were conducted by centrally trained nurses and medical assistants following a standardized protocol.[19] Detailed procedures of clinical and laboratory examinations have been published.[19] Participants were asked to fast for 12 hours before blood samples were taken in the morning, at baseline and in the two subsequent visits. Spot urine samples were also collected in the morning and were frozen with 1 to 2 hours of collection. The biospecimens were stored at -70°C or lower before analyses.[19] Urine creatinine was measured by an automated alkaline picrate method. Specific gravity was measured with a Leica TS 400 total solid refractometer (Leica Microsystems, Buffalo, USA).

Kidney function measurements

Serum creatinine measures were conducted by a single core laboratory and determined by automated alkaline picrate methodology.[23] The estimated glomerular

filtration rate (eGFR) was estimated by using the Modification of Diet in Renal Disease Study equation:[24]

$$\text{eGFR (mL/min/1.73m}^2\text{)} = 186.3 \times (\text{Serum creatinine})^{-1.154} \times (\text{Age})^{-0.203} \times 0.742 \text{ (if female)} \times 1.210 \text{ (if African-American)}$$

[not applicable in our study]

Urine albumin and creatinine were measured at the Laboratory of the National Institute of Diabetes and Digestive and Kidney Diseases, Epidemiology and Clinical Research Branch, Phoenix, AZ, by an automated nephelometric immunochemical procedure and an automated alkaline picrate methodology, respectively.[19] Urine albumin-to-creatinine ratio (UACR) was used to estimate 24-hour urine albumin excretion. Macroalbuminuria was defined as a UACR > 300 mg/g creatinine.[25]

CKD definitions

To date, an operative definition of incident CKD has not yet been explicitly defined.[26] In this study, incident CKD was defined by increasingly more specific criteria to evaluate the sensitivity of results to different outcome definitions. First, incident cases with a reduced eGFR were defined as an eGFR less than 60 ml/min/1.73m^2 at visit 2 or visit 3. Second, incident cases with a reduced and a declining eGFR were defined as an eGFR less than 60 ml/min/1.73m^2 and a drop in eGFR of at least 25% at visit 2 or visit 3 (called thereafter "impaired eGFR"). Third, incident cases with both impaired renal function and macroalbuminuria were defined as an eGFR less than 60 ml/min/1.73m^2 and a drop in eGFR of at least 25%, and urine albumin-creatinine ratio ≥ 300 mg/g creatinine.

Fourth, incident cases of renal failure were defined as a doubling in serum creatinine levels or progression to end-stage renal disease (ESRD).[27] ESRD was defined as a requirement for maintenance renal replacement therapy including both dialysis and transplantation.[28]

Urine arsenic measurements

The urine concentrations of arsenic species in Strong Heart Study population was stable over a 10-year follow up, reflecting stability in arsenic exposure and the appropriate of one single arsenic measure to represent long-term arsenic exposure.[29] Detailed analytic methods and associated quality control procedures for arsenic analysis have been described.[30] Arsenic species concentrations were determined by high-performance liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS) that served as the arsenic selective detector (Agilent 1100 HPLC and Agilent 7700x ICP-MS, Agilent Technologies, Santa Clara, California). Arsenic speciation could discriminate species directly related to inorganic arsenic exposure (arsenite, arsenate, monomethylarsonate [MMA], and dimethylarsinate [DMA]) from those related to organic arsenicals in seafood (arsenobetaine as an overall marker of seafood arsenicals), which are generally considered nontoxic.[31] The limit of detection (LOD) for total arsenic and for inorganic arsenic (arsenite plus arsenate), MMA, DMA, and arsenobetaine plus other arsenic cations was 0.1 μg/L. The percentages of participants with concentrations below the limit of detection were 0.03% for total arsenic, 5.2% for inorganic arsenic, 0.8% for MMA, 0.03% for DMA, and 2.1% for arsenobetaine plus other arsenic cations. For participants with arsenic species below the LOD, levels were imputed as the corresponding LOD divided by the square root of 2. An in-house

reference urine and the Japanese National Institute for Environmental Studies No. 18 Human Urine were analyzed together with the samples. Interassay coefficients of variation for total arsenic, inorganic arsenic, MMA, DMA and arsenobetaine for the in-house reference urine were 4.4%, 6.0%, 6.5%, 5.9%, and 6.5%, respectively.

Arsenic exposure and arsenic metabolism

We used the sum of urine inorganic arsenic (arsenite and arsenate) and methylated arsenic species (MMA and DMA) as the biomarker of inorganic arsenic exposure from multiple sources.[32-34] The urine arsenic concentrations were divided by urine creatinine levels to account for urine dilution-concentration and expressed as μg/g creatinine. Urine concentrations of arsenobetaine and other arsenic cations were very low (median 0.68; interquartile range 0.42 to 1.50 μg/g creatinine), confirming that seafood intake was low in this sample, and indicating that DMA mainly came from inorganic arsenic exposure.[35] We used the proportions of urine inorganic arsenic (arsenite and arsenate, iAs), MMA and DMA over the sum of inorganic and methylated species, expressed as iAs%, MMA%, and DMA%, to evaluate arsenic metabolism.

Statistical analyses

Urine concentrations of the sum of inorganic and methylated species were modeled as quartiles and as log-transformed concentrations to stabilize the variability for right-skewed variables to compare the 75th and 25th percentile (interquartile range, IQR). Arsenic metabolism (iAs%, MMA%, and DMA%) was modeled as per IQR increment.

The primary analyses used Cox proportional hazards modeling to quantify the relative hazard of incident kidney disease associated with arsenic exposure and arsenic metabolism.[36] The time scale for survival analysis was age, allowing to control over this

strong risk factor of chronic kidney disease. To handle left-truncation induced at time of enrollment and appropriately aligning risk sets on the age scale, the late entry method was conducted using age at baseline as the individual entry time. All proportional hazards models were adjusted for study site (using the stratified Cox procedure), 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, waist-hip ratio, hypertension, diabetes, and fasting glucose. We conducted stratified analysis to explore the consistency of the association between arsenic metabolism and incident CKD across categories of these demographic and comorbid factors.

Results

Follow-up and kidney outcomes of participants

From baseline through Dec 31, 1999, 474 (15.1 %) participants had reduced eGFR, 330 (10.5%) had reduced eGFR and a drop in eGFR of at least 25%, 206 (6.6%) had both impaired eGFR and macroalbuminuria, and 172 (5.5%) developed renal failure. Overall, median concentration of the sum of inorganic and methylated arsenic species in the urine was 11.2 μg/L (interquartile range, 6.7 to 19.6 μg/L). Urine arsenic concentrations were higher in participants from Arizona (median 15.1 μg/L), followed by North and South Dakota (12.7 μg/L) and Oklahoma (median 7.3 μg/L). The median (interquartile range) for iAs%, MMA% and DMA% was 8.3 (5.8 to 11.2), 13.9 (10.8 to 17.4) and 77.5 (71.7 to 82.5), respectively. Men, participants from North and South Dakota, current smokers and participants with lower body mass index had higher iAs% and MMA%, and correspondingly lower DMA%.

Arsenic exposure and chronic kidney disease

Compared with the overall population, baseline urine arsenic concentrations were higher among participants with incident kidney disease defined by reduced eGFR (definition 1), impaired eGFR (definition 2), impaired eGFR with macroalbuminuria (definition3), and renal failure (definition 4) (Table 1). The strength of the association between an interquartile range increase in baseline urine arsenic concentrations and kidney insufficiency increased from a fully adjusted hazard ratio of 1.07 (95% CI 0.90-1.29) for participants with low eGFR (definition 1) to 1.49 (95% CI 1.10-2.01) for participants with renal failure (definition 4) (Table 2, Model 4).

Arsenic metabolism and kidney disease

Compared with the overall population, participants with incident kidney disease had lower baseline iAs% and MMA% but had higher DMA% (Table 1). When modeling each arsenic metabolism biomarker one at a time in fully adjusted models, only the association with iAs% was statistically significant (Table 3). For each interquartile range increase in iAs%, the hazard ratio was 0.80 (95%CI 0.70 -0.92) for reduced eGFR, 0.81 (CI 0.69-0.95) for impaired eGFR, 0.71 (0.58-0.87) for impaired eGFR and macroalbuminuria, and 0.73 (0.58-0.92) for renal failure (Table 3, model 4).

When modeling arsenic metabolism by including two biomarkers in the model at the same time, the adjusted hazard ratio for reduced eGFR comparing an interquartile range increase in iAs% was 0.62 (95% CI 0.49-0.78) and 0.73 (0.63-0.85) when it substituted MMA% and DMA%, respectively. The adjusted hazard ratio for an interquartile range increase in MMA% was 1.81 (95% CI 1.36-2.40) and 1.23 (1.06-1.43) when it substituted iAs% and DMA%, respectively. The adjusted hazard ratio for an

interquartile range increase in DMA% was 1.86 (1.37-2.52) and 0.71 (0.56-0.90) when it substituted iAs% and MMA%, respectively (Table 4, model 4). These association patterns were consistent across all incident CKD definitions (Table 4). In dose-response analyses, increasing MMA% and DMA% were related to increased risk of renal impairment when they substituted iAs% (Figure 1).

The association between arsenic metabolism and incident CKD was similar by age, sex, study site, and the sum of inorganic and methylated arsenic concentrations. The association, however, was stronger in participants with obesity (Table 5).

Discussion

Exposure to low to moderate arsenic levels of inorganic arsenic, as measured in urine, was prospectively associated with the development of kidney diseases. A specific pattern of arsenic metabolism was also significantly associated with increased risk of kidney diseases. The more arsenic is metabolized into methylated arsenic species in the body, the higher risk of developing kidney disease. The hypothetical rationale underlying this observation is that the unchanged form of arsenic may be less likely deposited in the renal tissue compared to MMA and DMA or that higher iAs% may reflect the relative inefficient arsenic methylation capacity in the renal tissue. Differential toxicity toward renal injuries of each arsenic species is also important. However, the mechanism of arsenic-induced renal damage remains unknown. Whether the association is causal, from an epidemiological perspective, either primarily caused by direct renal damage (e.g. glomurular damage or tubulo-interstitial fibrosis)[2, 37] or secondary to the arsenic-induced

cardiovascular diseases or other systematic diseases (e.g. cardio-renal syndrome type 5)[38, 39] is still largely speculative.

Our study showed that urine arsenic concentration was significantly associated with incident CKD and the prospective associations are stronger with increasing outcome specificity (e.g., the hazard ratio increased from 1.07 for reduced GFR to 1.49 for renal failure). The consistent directionality and sequentially increasing effect size of the point estimates strengthened the causal inference between arsenic exposure and kidney disease as both information bias and possible reverse causality have been minimized by this assessment approach. Our finding is also consistent with previous studies published in Taiwan and Sri Lanka with low-moderate arsenic exposure.[40, 41]

Regarding arsenic metabolism, only a relatively small case-control study (125 cases and 229 controls) from Taipei, Taiwan reported an association between arsenic metabolism and chronic kidney disease in the current literature.[40] This hospital-based case-control study, also conducted in a population characterized by low arsenic exposure (arsenic levels in drinking water <10 μg/L), found no association between arsenic metabolism, as measured in urine, and CKD defined by eGFR< 60 ml/min/1.73m^2 lasting for at least 3 months[40]. However, among study participants with a relative lower plasma level of lycopene, they found a higher iAs%, MMA%, or DMA% was associated with CKD, although their model did not adjust for total arsenic levels. The discrepancy in findings between our study and the study from Taiwan may be due to the difference in study population, study design, sampling methods, and statistical approach. Our study is prospective, allowing us to examine the temporal relationship between baseline arsenic metabolism and incident CKD. The stability of the Strong Heart Study Cohort and the

high retention rate through the 10-year follow up also greatly controlled potential selection bias. Finally, our study finding is robust to changing the definition of kidney diseases.

The role of arsenic metabolism in human is considered to reflect both detoxication and bioactivation.[4, 42] The relative potencies of the arsenicals have been proposed as follows: $DMA^{3+} > MMA^{3+} \geq As^{3+}$ and $As^{5+} > MMA^{5+}$ and DMA $^{5+}$.[43-45] It is therefore challenging to predict the arsenic toxicity given the same arsenic exposure as each individual may have different capacity to metabolize arsenic. Each arsenic species may also have a different affinity for different tissues and different kinetics of deposition and redistribution in the body.[46] Similar to other methylation processes, the contributions of both genetic and environmental factors to the inter-individual variability of arsenic metabolism are widely acknowledged.[47] The interplay among arsenic metabolism, global DNA methylation and one-carbon metabolism may further elaborate the individual susceptibility to arsenic-related diseases ranging from cancer, cardiovascular diseases, to neurodegenerative disorders.[48-50] Our prospective study supported the critical role of arsenic metabolism in developing kidney disease, suggesting that the arsenic metabolism profile needs to be incorporated into risk assessment of arsenic exposure.

Strengths of this study include high-quality data collection and rigorous laboratory methods for measuring concentrations of urine arsenic species.[19, 30] Biomarkers of arsenic metabolism are not influenced by the variation in the dilution-concentration of urine samples. This study also had some limitations. Both urine arsenic concentrations and parameters of arsenic metabolism were measured once at baseline visit (1981-1991). However, the temporal stability of arsenic metabolism has been

confirmed in repeated samples over a 10-year period.[5, 29] The classifications of kidney disease are based on single measurement at the follow up visit 2(1993-1995) and visit 3(1998-1999). However, our findings were robust to different renal outcome definitions with high sensitivity and high specificity. Moreover, the associations were stronger for outcomes with increasing outcome specificity. Other limitations were potential information bias (for instance, the GFR estimation is not based on isotopic measurements) and over-adjustment (for example, hypertension, diabetes and fasting glucose may be in the causal pathway).

Conclusions

Both urine arsenic concentration and arsenic metabolism are independently and prospectively associated with the development of kidney diseases. Eliminating environmental arsenic exposure remains essential and further efforts to integrate the information regarding arsenic methylation capacity into arsenic risk assessment are warranted. Our study is just the very first step in investigating the public health implications of arsenic metabolism in kidney diseases. More research is needed to verify our results and further explore the pathogenesis of arsenic-related kidney injury from the viewpoints of systemic metabolism, epigenomics, and the interaction with other potential environmental nephrotoxicants.

Table 1. Characteristics of Strong Heart Study participants at baseline (1989-1991). [#]

Characteristics	Baseline n=3,143	Incident CKD (Definition 1) eGFR <60 ml/min/1.73m^2 (n=474)	Incident CKD (Definition 2) eGFR <60 ml/min/1.73m^2 & ≥ 25% drop (n=330)	Incident CKD (Definition 3) eGFR <60 ml/min/1.73m^2 & ≥ 25% drop & UACR ≥ 300 mg/g (n=206)	Incident CKD (Definition 4) doubling creatinine and ESRD (n=172)
Age, year	54.4 (49-61.2)	58 (52.4-64)	56.3 (50.4-63.1)	55.5 (49.8-61.8)	53.9 (48.8-59)
Male, n(%)	1,349 (42.9)	150 (31.7)	119 (36.1)	80 (38.8)	62 (36.1)
Location, n(%)					
Arizona	1,106 (35.2)	192 (40.5)	165 (50.0)	115 (55.8)	111 (64.5)
Oklahoma	965 (30.7)	143 (30.2)	79 (23.9)	38 (18.5)	25 (14.5)
North and South Dakota	1,072 (34.1)	139 (29.3)	86 (26.1)	53 (25.7)	36 (20.9)
Education, n(%)					
No high school	711 (22.6)	123 (26.0)	95 (28.8)	77 (37.4)	52 (30.2)
Some high school	749 (23.8)	118 (24.9)	88 (26.7)	50 (24.3)	47 (27.3)
High school or more	1,683 (53.6)	233 (49.2)	147 (44.6)	79 (38.4)	73 (42.4)
Smoking, n(%)					
Never	997 (31.7)	171 (36.1)	111 (33.6)	70 (34.0)	57 (33.1)
Former	1,057 (33.6)	168 (35.4)	122 (37.0)	73 (35.4)	70 (40.7)
Current	1,089 (34.7)	135 (28.5)	97 (29.4)	63 (30.6)	45 (26.2)
Alcohol ,n(%)					
Never	467 (14.9)	86 (18.1)	53 (16.1)	30 (14.6)	22 (12.8)
Former	1,274 (40.5)	215 (45.4)	147 (44.6)	96 (46.6)	81 (47.1)
Current	1,402 (44.6)	173 (36.5)	130 (39.4)	80 (38.8)	69 (40.1)
Body mass index	30.1 (26.6-34.5)	30.4 (27.0-34.3)	30.5 (27.4-34.7)	30.4 (27.3-34.3)	30.5 (27.4-34.8)
Waist-hip ratio	0.96 (0.91-0.99)	0.96 (0.93-1.0)	0.96 (0.93-1.0)	0.97 (0.94-1.0)	0.97 (0.93-1.0)
Waist circumference (cm)	104 (95-114)	106 (97-115)	106 (97-117)	106 (97-116)	106 (97-117)
Urine creatinine, g/L	1.2 (0.8-1.7)	1.1 (0.7-1.5)	1.0 (0.7-1.5)	0.91 (0.6-1.3)	0.9 (0.6-1.3)
Specific gravity	1.019 (1.015-	1.019 (1.014-1.024)	1.02 (1.015-1.026)	1.022 (1.016-1.029)	1.021 (1.017-1.030)

	1.024)				
Serum creatinine, mg/dL	0.8 (0.7-0.9)	0.9 (0.8-1.0)	0.8 (0.7-0.9)	0.8 (0.7-0.9)	0.8 (0.7-0.9)
eGFR , ml/min/1.73m^2	82.5 (75.7-94.6)	77.0 (67.8-90.0)	81.5 (73.9-93.3)	82.3 (72.9-94.8)	89.4 (77.5-107.4)
Hypertension, n(%)	1,070 (34.0)	217 (45.8)	150 (45.5)	97(52.9)	77(44.8)
Diabetes mellitus, n(%)	1,503 (47.8)	322 (67.9)	256 (77.6)	187 (90.8)	145 (84.3)
Fasting glucose, mg/dL$^{\Delta}$	115 (100-178)	159.5 (108-261)	189 (118-286)	243.0 (152-313)	229.5 (132-310)
HbA1c, % †	5.6 (5-8)	7.4 (5.4-10.5)	8.9 (5.6-11.0)	10.1 (7.6-11.3)	9.7 (6.0-11.3)
Arsenic exposure					
iAs + methylated arsenic*, μg/g	10.3 (6.1-16.3)	11.8 (6.6-18.5)	12.9 (7.6-20.4)	14.6 (8.5-22.3)	14.6 (10.2-23.6)
iAs, μg/g	0.8 (0.4-1.6)	0.8 (0.4-1.5)	1.0 (0.5-1.7)	1.1 (0.6-2.0)	1.2 (0.7-2.0)
MMA, μg/g	1.4 (0.8-2.3)	1.5 (0.9-2.6)	1.7 (1.0-2.8)	1.9 (1.1-3.0)	2.0 (1.3-3.0)
DMA, μg/g	7.7 (4.7-12.5)	9.2 (5.2-14.0)	10.0 (5.9-15.5)	11.1 (6.5-17.6)	11.2 (7.8-18.6)
Arsenic metabolism					
iAs%	8.3 (5.8-11.2)	7.1 (5.1-9.9)	7.5 (5.6-10.0)	7.6 (6.0-9.9)	7.8 (6.0-10.0)
MMA%	13.9 (10.8-17.4)	13.4 (10.5-16.3)	13.5 (10.5-16.4)	13.6 (10.5-16.4)	13.5 (10.7-16.3)
DMA%	77.5 (71.7-82.5)	79.2 (73.9-83.4)	78.5 (73.0-82.9)	78.4 (73.0-82.6)	78.4 (73.1-82.5)

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; UACR, urine albumin-creatinine ratio

$^{\#}$ Continuous variables are presented as the median (interquartile range)

$^{\Delta}$n for fasting glucose is 3,137; †n for HbA1c is 2,969, 444, 309, 189 and 161 for baseline, eGFR<60 ml/min/1.73m^2, eGFR<60 ml/min/1.73m^2 & $\geq$ 25% drop, eGFR<60 ml/min/1.73m^2 & $\geq$ 25% drop & UACR $\geq$ 300 mg/g, and renal failure subgroups, respectively.

Table 2. Hazard ratios (95% confidence intervals) for incident kidney disease per interquartile range in urine concentrations of inorganic arsenic (iAs), monomethylarsonate (MMA), dimethylarsinate (DMA) and the sum of iAs, MMA and DMA (μg/L) adjusted by urine creatinine (log-transformed).

Arsenic concentrations (interquartile range)	Model 1	Model 2	Model 3	Model 4	Model 5
eGFR <60 ml/min/1.73m^2					
iAs (0.5-1.8 μg/L)	0.94 (0.80-1.11)	0.92 (0.78-1.09)	0.91 (0.77-1.07)	0.85 (0.72-1.01)	0.89 (0.76-1.04)
MMA (0.8-2.8 μg/L)	1.13 (0.95-1.34)	1.13 (0.95-1.34)	1.15 (0.96-1.37)	1.10 (0.92-1.32)	1.04 (0.90-1.20)
DMA (5.2-14.7 μg/L)	1.25 (1.06-1.47)	1.23 (1.04-1.45)	1.17 (0.99-1.38)	1.09 (0.92-1.29)	1.05 (0.92-1.19)
iAs + methylated arsenic (6.7-19.6 μg/L)	1.22 (1.03-1.45)	1.20 (1.01-1.43)	1.15 (0.97-1.38)	1.07 (0.90-1.29)	1.04 (0.91-1.19)
eGFR <60 ml/min/1.73m^2 & ≥ 25% drop					
iAs (0.5-1.8 μg/L)	1.01 (0.83-1.23)	0.99 (0.81-1.22)	0.96 (0.78-1.17)	0.88 (0.72-1.09)	0.88 (0.73-1.05)
MMA (0.8-2.8 μg/L)	1.21 (0.98-1.48)	1.21 (0.99-1.50)	1.23 (1.00-1.52)	1.16 (0.93-1.44)	1.01 (0.85-1.20)
DMA (5.2-14.7 μg/L)	1.38 (1.14-1.68)	1.35 (1.11-1.64)	1.26 (1.03-1.54)	1.15 (0.94-1.42)	1.01 (0.87-1.18)
iAs + methylated arsenic (6.7-19.6 μg/L)	1.35 (1.10-1.65)	1.32 (1.08-1.62)	1.25 (1.01-1.54)	1.14 (0.92-1.42)	1.00 (0.85-1.18)
eGFR <60 ml/min/1.73m^2 & ≥ 25% drop & UACR ≥ 300 mg/g					
iAs (0.5-1.8 μg/L)	1.09 (0.85-1.39)	1.06 (0.82-1.37)	1.00 (0.77-1.29)	0.87 (0.67-1.14)	0.86 (0.69-1.08)
MMA (0.8-2.8 μg/L)	1.39 (1.08-1.79)	1.39 (1.07-1.81)	1.40 (1.07-1.84)	1.27 (0.96-1.68)	1.03 (0.83-1.27)
DMA (5.2-14.7 μg/L)	1.64 (1.28-2.09)	1.62 (1.26-2.07)	1.45 (1.12-1.87)	1.23 (0.94-1.61)	1.02 (0.84-1.24)
iAs + methylated arsenic (6.7-19.6 μg/L)	1.58 (1.22-2.03)	1.56 (1.21-2.02)	1.41 (1.08-1.85)	1.20 (0.91-1.59)	1.00 (0.82-1.23)
Renal failure					
iAs (0.5-1.8 μg/L)	1.21 (0.92-1.59)	1.20 (0.91-1.59)	1.15 (0.87-1.53)	1.02 (0.76-1.37)	1.00 (0.77-1.28)
MMA (0.8-2.8 μg/L)	1.62 (1.23-2.14)	1.64 (1.24-2.18)	1.66 (1.24-2.22)	1.51 (1.12-2.04)	1.20 (0.94-1.52)
DMA (5.2-14.7 μg/L)	1.84 (1.41-2.40)	1.83 (1.40-2.40)	1.70 (1.29-2.26)	1.50 (1.12-2.01)	1.17 (0.94-1.46)
iAs + methylated arsenic (6.7-19.6 μg/L)	1.80 (1.37-2.37)	1.80 (1.36-2.38)	1.69 (1.27-2.26)	1.49 (1.10-2.01)	1.17 (0.93-1.47)

Model 1: Stratified by study center and adjusted for age (age as time metric and age at baseline were treated as staggered entries), urine creatinine (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 diabetes and hypertension

Model 4: Further adjusted fasting glucose

Model 5: Without adjust urine creatinine

Table 3. Hazard ratios (95% confidence intervals) for incident kidney disease per interquartile range in arsenic metabolism biomarkers (the % of inorganic arsenic [iAs%], the % of monomethylarsonate [MMA%] and the % of dimethylarsinate [DMA%]). One biomarker was entered into model as a main predictor each time and all models adjusted for the urine concentrations of sum of iAs, MMA and DMA corrected by urine creatinine.

Arsenic metabolism (interquartile range)		**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
eGFR <60 ml/min/1.73m^2						
iAs%	(5.8-11.2)	0.78 (0.68-0.89)	0.78 (0.68-0.90)	0.80 (0.70-0.91)	0.80 (0.70-0.92)	0.82 (0.72-0.94)
MMA%	(10.8-17.4)	0.95 (0.83-1.08)	0.97 (0.84-1.11)	1.04 (0.91-1.20)	1.07 (0.93-1.22)	1.07 (0.93-1.22)
DMA%	(71.7-82.5)	1.19 (1.04-1.36)	1.18 (1.02-1.36)	1.11 (0.97-1.28)	1.09 (0.95-1.25)	1.08 (0.94-1.25)
eGFR <60 ml/min/1.73m^2 & ≥ 25% drop						
iAs%	(5.8-11.2)	0.79 (0.67-0.92)	0.79 (0.67-0.92)	0.80 (0.69-0.94)	0.81 (0.69-0.95)	0.85 (0.73-0.99)
MMA%	(10.8-17.4)	0.92 (0.79-1.08)	0.95 (0.81-1.12)	1.05 (0.89-1.23)	1.08 (0.92-1.26)	1.07 (0.91-1.26)
DMA%	(71.7-82.5)	1.21 (1.03-1.42)	1.19 (1.00-1.40)	1.11 (0.94-1.31)	1.08 (0.92-1.28)	1.06 (0.90-1.25)
eGFR <60 ml/min/1.73m^2 & ≥ 25% drop & UACR ≥ 300 mg/g						
iAs%	(5.8-11.2)	0.67 (0.55-0.82)	0.67 (0.55-0.82)	0.69 (0.57-0.85)	0.71 (0.58-0.87)	0.75 (0.61-0.91)
MMA%	(10.8-17.4)	0.93 (0.76-1.12)	0.94 (0.77-1.15)	1.08 (0.89-1.32)	1.13 (0.93-1.38)	1.13 (0.93-1.38)
DMA%	(71.7-82.5)	1.32 (1.07-1.61)	1.32 (1.07-1.63)	1.18 (0.96-1.46)	1.13 (0.92-1.39)	1.10 (0.90-1.36)
Renal failure						
iAs%	(5.8-11.2)	0.71 (0.57-0.89)	0.71 (0.57-0.89)	0.73 (0.58-0.91)	0.73 (0.58-0.92)	0.79 (0.63-0.98)
MMA%	(10.8-17.4)	0.95 (0.77-1.19)	0.97 (0.77-1.21)	1.07 (0.86-1.34)	1.10 (0.88-1.37)	1.10 (0.88-1.38)
DMA%	(71.7-82.5)	1.26 (1.00-1.57)	1.26 (0.99-1.58)	1.16 (0.92-1.46)	1.13 (0.89-1.43)	1.09 (0.86-1.38)

Model 1: Stratified by study center and adjusted for age (age as time metric and age at baseline were treated as staggered entries), urine arsenic concentration (log-transformed), urine creatinine (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 diabetes and hypertension
Model 4: Further adjusted fasting glucose
Model 5: Without adjust urine creatinine

Table 4. Hazard ratios (95% confidence intervals) for incident kidney disease per interquartile range in arsenic metabolism biomarkers (the % of inorganic arsenic [iAs], the % of monomethylarsonate [MMA] and the % of dimethylarsinate [DMA]). As the three biomarkers equal 100%, models entered two biomarkers at a time. All models adjusted for the urine concentrations of sum of iAs, MMA and DMA corrected by urine creatinine.

Arsenic metabolism (interquartile range)	Model 1	Model 2	Model 3	Model 4	Model 5
eGFR <60 ml/min/1.73m^2					
iAs% substituted by:					
MMA% (10.8-17.4)	1.54 (1.16-2.05)	1.58 (1.19-2.11)	1.77 (1.33-2.36)	1.81 (1.36-2.40)	1.69 (1.28-2.24)
DMA% (71.7-82.5)	1.77 (1.31-2.39)	1.78 (1.32-2.42)	1.86 (1.37-2.52)	1.86 (1.37-2.52)	1.73 (1.29-2.32)
MMA% substituted by:					
iAs% (5.8-11.2)	0.70 (0.56-0.89)	0.69 (0.54-0.87)	0.63 (0.50-0.79)	0.62 (0.49-0.78)	0.65 (0.52-0.82)
DMA% (71.7-82.5)	0.87 (0.69-1.11)	0.85 (0.66-1.08)	0.74 (0.58-0.94)	0.71 (0.56-0.90)	0.73 (0.58-0.93)
DMA% substituted by:					
iAs% (5.8-11.2)	0.75 (0.65-0.88)	0.75 (0.64-0.87)	0.73 (0.63-0.85)	0.73 (0.63-0.85)	0.76 (0.66-0.88)
MMA% (10.8-17.4)	1.09 (0.94-1.26)	1.11 (0.95-1.29)	1.21 (1.04-1.40)	1.23 (1.06-1.43)	1.21 (1.04-1.40)
eGFR <60 ml/min/1.73m^2 & $\geq$ 25% drop					
iAs% substituted by:					
MMA% (10.8-17.4)	1.44 (1.03-2.02)	1.52 (1.08-2.14)	1.76 (1.26-2.47)	1.82 (1.30-2.53)	1.64 (1.17-2.29)
DMA% (71.7-82.5)	1.69 (1.19-2.40)	1.73 (1.21-2.46)	1.83 (1.29-2.61)	1.84 (1.29-2.62)	1.64 (1.16-2.31)
MMA% substituted by:					
iAs% (5.8-11.2)	0.74 (0.56-0.97)	0.71 (0.54-0.94)	0.63 (0.48-0.83)	0.62 (0.47-0.81)	0.67 (0.51-0.88)
DMA% (71.7-82.5)	0.93 (0.70-1.23)	0.87 (0.65-1.17)	0.73 (0.54-0.97)	0.70 (0.52-0.93)	0.73 (0.55-0.98)
DMA% substituted by:					
iAs% (5.8-11.2)	0.77 (0.65-0.92)	0.76 (0.64-0.91)	0.74 (0.62-0.88)	0.74 (0.62-0.88)	0.78 (0.66-0.93)
MMA% (10.8-17.4)	1.05 (0.88-1.25)	1.09 (0.91-1.31)	1.22 (1.02-1.45)	1.25 (1.05-1.49)	1.21 (1.01-1.45)
eGFR <60 ml/min/1.73m^2 & ≥ 25% drop & UACR ≥ 300 mg/g					
iAs% substituted by:					
MMA% (10.8-17.4)	1.98 (1.32-2.98)	2.06 (1.36-3.11)	2.34 (1.60-3.44)	2.40 (1.65-3.49)	2.23 (1.51-3.29)

DMA%	(71.7-82.5)	2.47 (1.58-3.87)	2.53 (1.62-3.98)	2.56 (1.66-3.95)	2.51 (1.64-3.85)	2.25 (1.47-3.46)
MMA% substituted by:						
iAs%	(5.8-11.2)	0.57 (0.41-0.80)	0.56 (0.40-0.78)	0.50 (0.37-0.68)	0.49 (0.36-0.67)	0.52 (0.38-0.71)
DMA%	(71.7-82.5)	0.81 (0.58-1.14)	0.78 (0.55-1.11)	0.64 (0.46-0.89)	0.61 (0.44-0.83)	0.61 (0.44-0.86)
DMA% substituted by:						
iAs%	(5.8-11.2)	0.64 (0.51-0.80)	0.63 (0.50-0.79)	0.62 (0.50-0.78)	0.63 (0.51-0.78)	0.67 (0.54-0.82)
MMA%	(10.8-17.4)	1.14 (0.92-1.40)	1.16 (0.94-1.44)	1.31 (1.07-1.61)	1.36 (1.12-1.66)	1.35 (1.10-1.67)
Renal failure						
iAs% substituted by:						
MMA%	(10.8-17.4)	1.86 (1.19-2.91)	1.88 (1.20-2.95)	2.09 (1.36-3.20)	2.11 (1.39-3.21)	1.90 (1.22-2.96)
DMA%	(71.7-82.5)	2.22 (1.37-3.59)	2.22 (1.36-3.60)	2.25 (1.40-3.63)	2.23 (1.39-3.59)	1.93 (1.20-3.11)
MMA% substituted by:						
iAs%	(5.8-11.2)	0.60 (0.42-0.87)	0.60 (0.41-0.86)	0.55 (0.39-0.78)	0.54 (0.39-0.77)	0.59 (0.41-0.85)
DMA%	(71.7-82.5)	0.81 (0.55-1.17)	0.79 (0.54-1.17)	0.68 (0.47-0.98)	0.66 (0.46-0.95)	0.68 (0.46-1.00)
DMA% substituted by:						
iAs%	(5.8-11.2)	0.67 (0.53-0.86)	0.67 (0.53-0.86)	0.67 (0.53-0.85)	0.70 (0.53-0.85)	0.72 (0.57-0.91)
MMA%	(10.8-17.4)	1.14 (0.91-1.44)	1.15 (0.91-1.46)	1.27 (1.01-1.59)	1.29 (1.03-1.61)	1.27 (1.00-1.61)

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

Model 4: Further adjusted fasting glucose

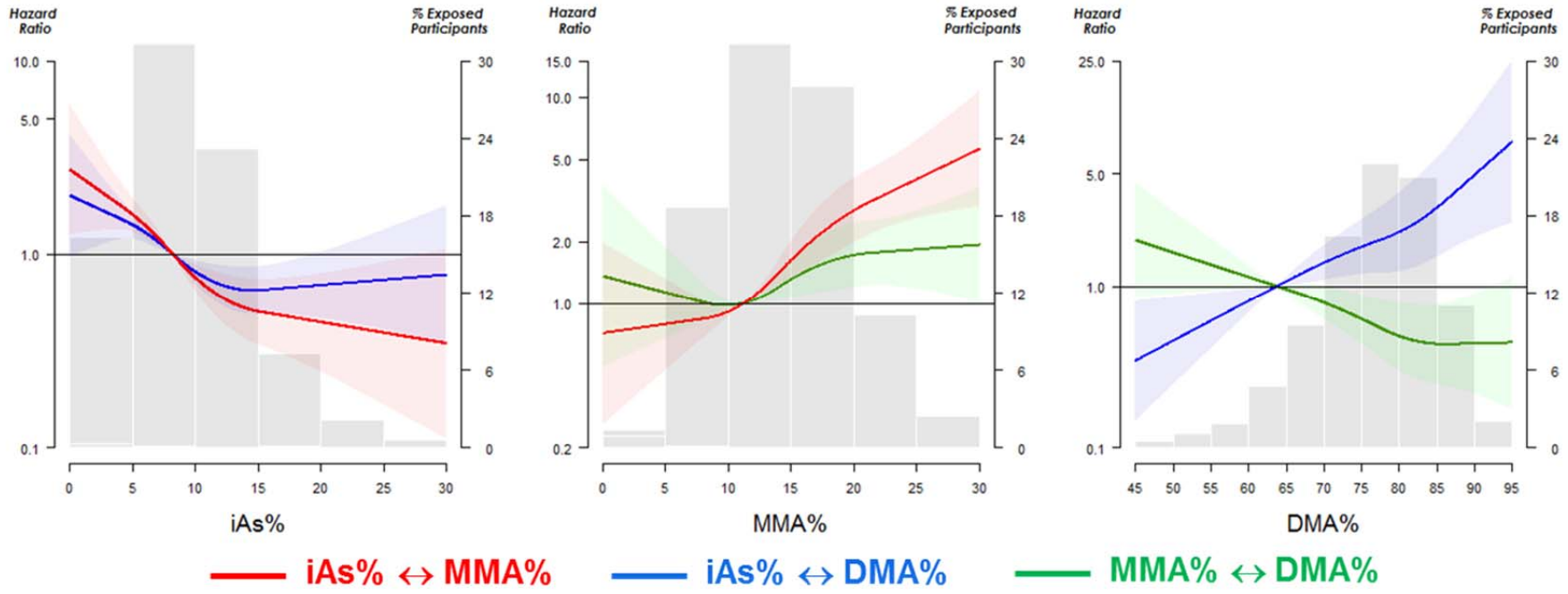
Model 5: Without adjust urine creatinine

Table 5. Adjusted hazard ratios and 95% confidence intervals for incident kidney disease (definition 4) comparing the 75th with 25th percentile of the % of inorganic arsenic [iAs%], the % of monomethylarsonate [MMA%] and the % of dimethylarsonate[DMA%], by participant characteristics at baseline.

Subgroup	n	iAs% Substituted by		MMA% Substituted by		DMA% Substituted by	
		MMA%	DMA%	iAs%	DMA%	iAs%	MMA%
Age (years)							
< 55	1629	2.42 (1.48-3.96)	2.32 (1.26-4.27)	0.49 (0.33-0.73)	0.55 (0.36-0.85)	0.66 (0.48-0.89)	1.44 (1.10-1.89)
≥ 55	1484	2.30 (1.24-4.25)	2.55 (1.34-4.87)	0.51 (0.31-0.84)	0.66 (0.40-1.10)	0.63 (0.45-0.86)	1.29 (0.94-1.77)
p-value for interaction		0.75	0.54	0.75	0.86	0.54	0.86
Sex							
Men	1334	2.09 (1.11-3.93)	2.42 (1.44-4.08)	0.55 (0.33-0.92)	0.64 (0.37-1.12)	0.69 (0.50-0.94)	1.32 (0.93-1.86)
Women	1779	2.13 (1.13-4.00)	2.87 (1.52-5.43)	0.49 (0.32-0.74)	0.68 (0.45-1.04)	0.59 (0.43-0.81)	1.27 (0.97-1.64)
p-value for interaction		0.81	0.81	0.81	0.45	0.81	0.45
Study site							
Arizona	1094	3.30 (1.98-5.49)	4.19 (2.24-7.84)	0.38 (0.25-0.57)	0.60 (0.40-0.91)	0.49 (0.36-0.67)	1.37 (1.06-1.76)
Oklahoma	960	1.83 (0.61-5.47)	1.84 (0.57-5.93)	0.61 (0.25-1.49)	0.69 (0.29-1.61)	0.74 (0.41-1.32)	1.26 (0.75-2.12)
North/South Dekota	1059	1.53 (0.72-3.25)	1.16 (0.56-2.41)	0.71 (0.38-1.31)	0.58 (0.28-1.20)	0.93 (0.64-1.34)	1.40 (0.89-2.18)
p-value for interaction		0.38	0.08	0.38	0.99	0.08	0.99
Smoking							
Never	983	1.99 (0.97-4.05)	2.70 (1.14-6.39)	0.57 (0.32-1.02)	0.88 (0.49-1.59)	0.61 (0.40-0.94)	1.08 (0.75-1.55)
Former	1052	5.62 (2.58-12.25)	5.13 (2.20-11.94)	0.25 (0.13-0.46)	0.31 (0.17-0.57)	0.44 (0.29-0.67)	2.06 (1.41-3.01)
Current	1078	1.98 (0.91-4.31)	1.83 (0.85-3.91)	0.57 (0.30-1.08)	0.60 (0.30-1.22)	0.74 (0.51-1.08)	1.37 (0.89-2.11)
p-value for interaction		0.16	0.41	0.16	0.06	0.41	0.06
Obesity							
BMI< 30 kg/m^2	1498	1.75 (1.04-2.95)	1.70 (0.97-2.97)	0.63 (0.41-0.97)	0.68 (0.43-1.10)	0.77 (0.58-1.02)	1.26 (0.95-1.69)
BMI≥ 30 kg/m^2	1615	6.58 (3.19-13.58)	8.64 (3.81-19.60)	0.22 (0.12-0.39)	0.40 (0.23-0.70)	0.34 (0.23-0.51)	1.75 (1.25-2.45)
p-value for interaction		0.03	<0.01	0.03	0.43	<0.01	0.43

Waist-hip ratio							
Non-abdominal obesity*	892	2.58 (1.05-6.36)	2.63 (1.07-6.42)	0.46 (0.22-0.96)	0.56 (0.26-1.23)	0.62 (0.39-0.97)	1.42 (0.88-2.31)
Abdominal obesity	2221	2.33 (1.50-3.63)	2.47 (1.47-4.14)	0.50 (0.35-0.72)	0.62 (0.43-0.90)	0.64 (0.49-0.82)	1.34 (1.06-1.69)
p-value for interaction		0.61	0.55	0.61	0.82	0.55	0.82
iAs+MMA+DMA							
≤ 5.5 μg/g	782	2.10 (0.49-8.99)	1.61 (0.37-7.09)	0.55 (0.17-1.79)	0.48 (0.16-1.44)	0.79 (0.38-1.65)	1.57 (0.80-3.06)
> 5.5 & ≤ 8.8 μg/g	780	2.50 (1.06-5.89)	1.74 (0.65-4.69)	0.47 (0.24-0.95)	0.39 (0.18-0.86)	0.76 (0.46-1.24)	1.78 (1.10-2.88)
>8.8 & ≤ 13.9 μg/g	775	3.56 (1.11-11.43)	4.00 (1.28-12.47)	0.36 (0.14-0.92)	0.51 (0.19-1.36)	0.50 (0.28-0.88)	1.52 (0.83-2.79)
> 13.9 μg/g	776	1.85 (1.04-3.31)	2.20 (1.11-4.34)	0.61 (0.38-0.97)	0.81 (0.49-1.33)	0.67 (0.48-0.95)	1.14 (0.84-1.56)
p-value for interaction		0.78	0.96	0.78	0.17	0.96	0.17
Overall	3113	2.40 (1.65-3.49)	2.51 (1.64-3.85)	0.49 (0.36-0.67)	0.61 (0.44-0.83)	0.63 (0.51-0.78)	1.36 (1.12-1.66)

Figure 1. Hazard ratios for incident kidney disease (definition 2) 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% (blue line) in the middle panel and the hazard ratio for DMA% when it replaces iAs% (red line) and MMA% (blue line). The shaded areas represent 95% CIs.



SYNTHESIS

Introduction

This dissertation describes the role of arsenic metabolism in a broad spectrum of health conditions including all-cause mortality, cardiovascular disease mortality, cancer mortality, incident diabetes, and kidney diseases based on population-based prospective cohort data. Our study is the first to show the dynamic relationship among iAs%, MMA%, and DMA%. The data support the hypothesis that certain profiles of arsenic metabolism are associated with different chronic diseases. Previous evidence, conducted mostly in Taiwan[1-3], found that higher MMA% and lower DMA% in urine were related to increased risk of developing cancer and cardiovascular diseases while higher DMA% and lower MMA% were associated with the risk of obesity and diabetes.[4-7] Through systematic statistical modeling, we have further advanced the understanding of the mutual dynamics among biomarkers of arsenic metabolism and provide important information for risk assessment and risk management of arsenic toxicity. This chapter summarizes the results of these projects and discusses the implications of the data.

Summary of findings

In the first chapter, we conducted a systematic review to identify areas of knowledge gap in the risk assessment of arsenic metabolism in different disease outcomes including cancer, cardiovascular disease, obesity, and diabetes. For cancer, although many studies supported that higher MMA% and lower DMA% were associated with the development of cancer especially for urothelial cancer, these studies were relatively small and most of them were conducted in Taiwan using hospital-based case-

control study designs.[2] Furthermore, the associations were mostly detected in subgroup analyses (interaction analyses) and most prospective studies did not support this hypothesis.[8-10] The role of arsenic metabolism in cardiovascular diseases remains unclear and conflicting. However, two prospective cohort studies from Taiwan and Bangladesh showed higher MMA% was associated with carotid atherosclerosis and cardiovascular diseases, respectively.[11, 12] Few studies examined the association between arsenic metabolism and obesity and diabetes.[5, 6, 13, 14] Decreasing MMA% was consistently linked to the increasing body mass index. However, for diabetes, no specific pattern of arsenic methylation capacity was identified in the current literature although very few studies have looked at this question and their sample size is relatively small. In addition, the statistical modeling of arsenic metabolism was highly heterogeneous across the enrolled studies and was not appropriate as all studies ignored the compositional nature of arsenic metabolism, which made interpretation and application difficult and prevented meaningful statistical inference.

In the second chapter, we examined the relationship between arsenic metabolism and all-cause mortality, CVD mortality, and cancer mortality in 3,600 adults 45-75 years old in American Indian communities from the three centers that participated in the Strong Heart Study in the US at the baseline visit in 1989-1991 and had completed information on urine concentrations of inorganic arsenic and methylated arsenic species. Vital status and cause-of-death codes were determined by annual contact, review of hospitalization records and death certificates, and information obtained from National Death Index. Through a median follow-up of 17.3 years, 1,559 (43.3%) participants died of any cause, 484 (13.4 %) died of cardiovascular disease (CVD), and 281 (7.8%) died of cancer.

Overall, median concentration of the sum of inorganic and methylated arsenic species in the urine was 11.2 μg/L (interquartile range, 6.6 to 19.1μg/L). Urine arsenic concentrations were higher in participants from Arizona (median 14.9 μg/L), followed by the North and South Dakotas (12.6 μg/L) and Oklahoma (median 7.2 μg/L). The median (interquartile range) for inorganic arsenic%, MMA% and DMA% was 8.0 (5.6 to 11.0), 14.0 (10.8 to 17.6) and 77.7 (71.9 to 82.6), respectively. In multi-adjusted Cox proportional hazards model, we found the substitution of iAs% by DMA% was prospectively associated with higher all-cause mortality. The substitution of iAs% by either MMA% or DMA% was associated with higher cardiovascular disease (CVD) mortality. The substitution of DMA% by MMA% was also related to higher CVD mortality. For cancer mortality, the substitution of MMA% by either iAs% or DMA% was prospectively associated with higher cancer mortality. In addition, we found no significant interaction between urine arsenic concentrations and biomarkers of arsenic metabolism.

In the third chapter, we examined the relationship between arsenic metabolism and incident diabetes in 1,694 diabetes-free adults 45-75 years old in American Indian communities from the three centers that participated in the Strong Heart Study in the US at the baseline visit in 1989-1991 and had completed information on urine concentrations of inorganic arsenic and methylated arsenic species. Diabetes was defined as a fasting plasma glucose ≥126 mg/dL, venous plasma glucose 2–h after ingestion of 75 g oral glucose load ≥200 mg/dL, self-reported diabetes history, and self-reported use of insulin or oral hypoglycemic medications. The median urine concentration of the inorganic arsenic plus methylated arsenic species was 10.2 μg/L creatinine (interquartile range, 6.1

to 17.7 μg/L). Urine arsenic concentrations were higher in participants from Arizona (median 14.3 μg/L), followed by the Dakotas (11.9 μg/L) and Oklahoma (median 7.0 μg/L). The median (interquartile range) for inorganic arsenic%, MMA% and DMA% was 8.3 (5.7 to 11.3), 15.2 (11.7 to 18.8) and 76.4 (70.3 to 81.4), respectively. Over 11,263.2 person-years of follow-up, 396 participants developed diabetes. Diabetes incidence was 35.2 per 1000 person-years. In multi-adjusted Cox proportional hazards model, we found that higher MMA% in urine, either because of lower inorganic arsenic% or lower DMA%, was associated with lower incidence of diabetes. We found no significant interaction between urine arsenic concentrations and biomarkers of arsenic metabolism.

In the fourth chapter, we examined the relationship between arsenic metabolism and incident kidney disease in 3,143 adults 45-75 years old with normal renal function in American Indian communities from the three centers that participated in the Strong Heart Study in the US at the baseline visit in 1989-1991 and had completed information on urine concentrations of inorganic arsenic and methylated arsenic species. Incident CKD was defined by criteria with increasing specificity to evaluate the sensitivity of the results to different outcome definitions. First, we defined reduced eGFR as an eGFR less than 60 ml/min/1.73m^2. Second, we defined impaired eGFR as an eGFR less than 60 ml/min/1.73m^2 and a drop in eGFR of at least 25%. Third, we defined impaired renal function and macroalbuminuria as an eGFR less than 60 ml/min/1.73m^2 with a drop in eGFR of at least 25% and urine albumin-creatinine ratio $\geq$ 300 mg/g creatinine. Fourth, renal failure was measured by doubling serum creatinine level or progression to end-stage renal disease (ESRD). In multi-adjusted Cox proportional hazards model, the hazard ratio of incident kidney disease defined by low eGFR (definition 1) for an interquartile range

increase in iAs% was 0.62 (95% CI 0.49-0.78) and 0.73 (0.63-0.85) when it substituted MMA% and DMA%, respectively. The results were robust to different incident CKD definitions. The effects of replacing iAs% by MMA% or DMA% on the risk of developing CKD displayed a linear dose-response relationship and were enhanced among obese participants defined by body mass index higher than 30 kg/m^2. The risk patterns of urine arsenic metabolism profiles can be summarized as follows: 1) When the proportions of methylated arsenic species increased with a corresponding decrease of the proportion of inorganic arsenic, the risk of all-cause and cardiovascular mortality and kidney diseases increased. 2) When the proportion of monomethylated arsenic increased with a corresponding decrease of the proportion of either inorganic arsenic or dimethylated arsenic, the risk of cancer mortality and incident diabetes decreased (Figure 1).

Implications and Future Research

Our data support that specific patterns of arsenic metabolism are significantly associated with the risk of mortality, diabetes, and kidney disease. Profiles of arsenic metabolism reflect inter-individual difference in arsenic methylation capacity and may represent the overall effect of host-environmental interaction on arsenic toxicities (figure 2). Our findings further support the theory that arsenic methylation may be a bio-activation process rather than just detoxication and also support that different arsenic species may have different tissue affinities and pathogenic mechanisms underlying different disease phenotypes. Before applying our findings to arsenic risk assessment and risk management, Bradford Hill's causation criteria provides a framework for us to identify the gaps and needs to establish causality and inform future research.

1. **Strength of Association.**

   For per interquartile increase in a specific marker of arsenic metabolism that replaces two other markers, the hazard ratio for all-cause mortality, cause-specific mortality, incident diabetes, and incident kidney disease ranged from 1.04 to 1.83. This is consistent with a moderate association.

2. **Temporality and consistency**

   The prospective evidence linking arsenic metabolism and mortality, diabetes, and kidney disease is scarce. Our findings are not consistent with previous literature regarding diabetes and kidney disease.[5, 15] However, previous studies were relatively small and very few were prospective cohort study designs, making evidence interpretation difficult. To establish the consistency of our findings, future research is needed to replicate our findings in different populations with a larger sample size and consistent statistical modeling to facilitate subsequent meta-analysis.

3. **Biological gradient**

   In dose-response analysis, we found certain markers of arsenic metabolism are linearly associated with the risk of certain endpoints, for instance, with chronic kidney disease. However, for all-cause mortality, the relationship was close to a threshold dose response.

4. **Specificity**

   Our research is not able to support the specificity. However, relevant research conducted in Taiwan and Bangladesh may have good opportunities to inform on specificity. For instance, Gamble and associate found folate could modify the

profile of arsenic metabolism; however, long-term effects of arsenic metabolism modification remain unknown.[16] In Taiwan, water intervention has been implemented for 30 years, a careful follow-up of the change in arsenic metabolism and the prevalence/incidence of various diseases can inform the specificity of arsenic metabolism.[17] Further investigation in this important topic is critical to establish a risk management framework of arsenic.

5. Plausibility, coherence, and experiments

a. Our findings are coherent with the bioactivation theory of arsenic methylation supported by the fact that methylated trivalent arsenicals are highly toxic.[18-22] MMA(III) is especially of interest to researchers and has been considered more potent than arsenite from the perspective of cytotoxicity and genotoxicity.[23, 24] Recently, DMA(III) was also linked to the risk of diabetes in recent cross-sectional studies conducted in Mexico. However, systematic evidence evaluating the risk of arsenic species remain lacking mainly due to absence of stable measurements of MMA(III) and DMA(III) and the lack of standardized methods to adjust for differences in urine dilution-concentration especially at low level arsenic exposure. In contrast, markers of arsenic metabolism (iAs%, MMA%, and DMA%) provide an unique opportunity to model individual arsenic methylation capacity and get rid of the issues related to urine dilution correction. However, the main challenge of adopting markers of arsenic metabolism in risk assessment is the data interpretation, as the pathogenesis underlying different patterns of arsenic methylation capacity

is unknown. Our research motivates the need to conduct more epidemiological and experimental research to study the mechanisms underlying the risk patterns of arsenic metabolism. For instance, whether the individual methylation capacity of arsenic is linked to the efficiency of other methylation reactions remains unknown. Increasing evidence has shown a tight interconnection with one-carbon metabolism; however, other metabolic pathways may also interplay with arsenic metabolism.[25] In addition, few evidence studying the genetic and environmental determinants of arsenic metabolism in human, more research is urgently needed for this fundamental question to assist practical risk management.

b. On the other hand, as the markers of arsenic methylation capacity may represent certain genetic effects on arsenic toxicity, we may use metabolism profiles as an instrument variable to control unknown confounders and maximize our ability to identify causal effects.

c. The patterns of arsenic metabolism associated with cardiovascular mortality and the risk of developing kidney disease are close to each other, supporting the perspective that cardiovascular and kidney diseases may share a common metabolic pathogenesis. On the other hand, the similar arsenic methylation profiles between cancer mortality and incident diabetes could be consistent with a common etiological link. These observations are coherent with current perspectives on the etiological connections between cardiovascular disease and kidney disease, and between diabetes and cancer, respectively.[26, 27]

6. **The role of arsenic metabolism in toxicological paradigm**

   Previous studies suggested arsenic methylation profiles are potential markers of host susceptibility to arsenic exposure based on mostly candidate gene association studies and exposure-biomarker interaction analyses. In this dissertation, the role of arsenic metabolism in the pathogenesis of mortality, diabetes, and kidney disease may be beyond susceptibility as we found no significant statistical interaction between arsenic exposure and arsenic metabolism. Moreover, arsenic metabolism may be an integrated biomarker of how individuals' susceptibility and vulnerability status respond to arsenic exposure resulting in different tissue retention, distribution and excretion and the patterns of this response may predispose individuals to various clinical outcomes (Figure 2).

Conclusion

Markers of arsenic metabolism are novel risk factors of all-cause mortality, cardiovascular mortality, cancer mortality, incident diabetes, and incident kidney diseases. Before applying these findings in risk assessment and risk management of arsenic toxicity, it would be critical to replicate our results in other populations with extensive range of arsenic exposure, with longer follow-up, and with larger sample size. At the policy level, in addition to implementing strict control of arsenic exposure, public health efforts may focus on risk stratification and patterns of arsenic metabolism may guide to conduct risk management and link environmental effects to individual health care. At the community and individual levels, we need to build and solidify public

awareness of the health risks of arsenic as there may be no safe zone regarding individual susceptibility toward arsenic.

Figure 1. Summary of the risk patterns of urine arsenic metabolism profiles with different clinical endpoints.

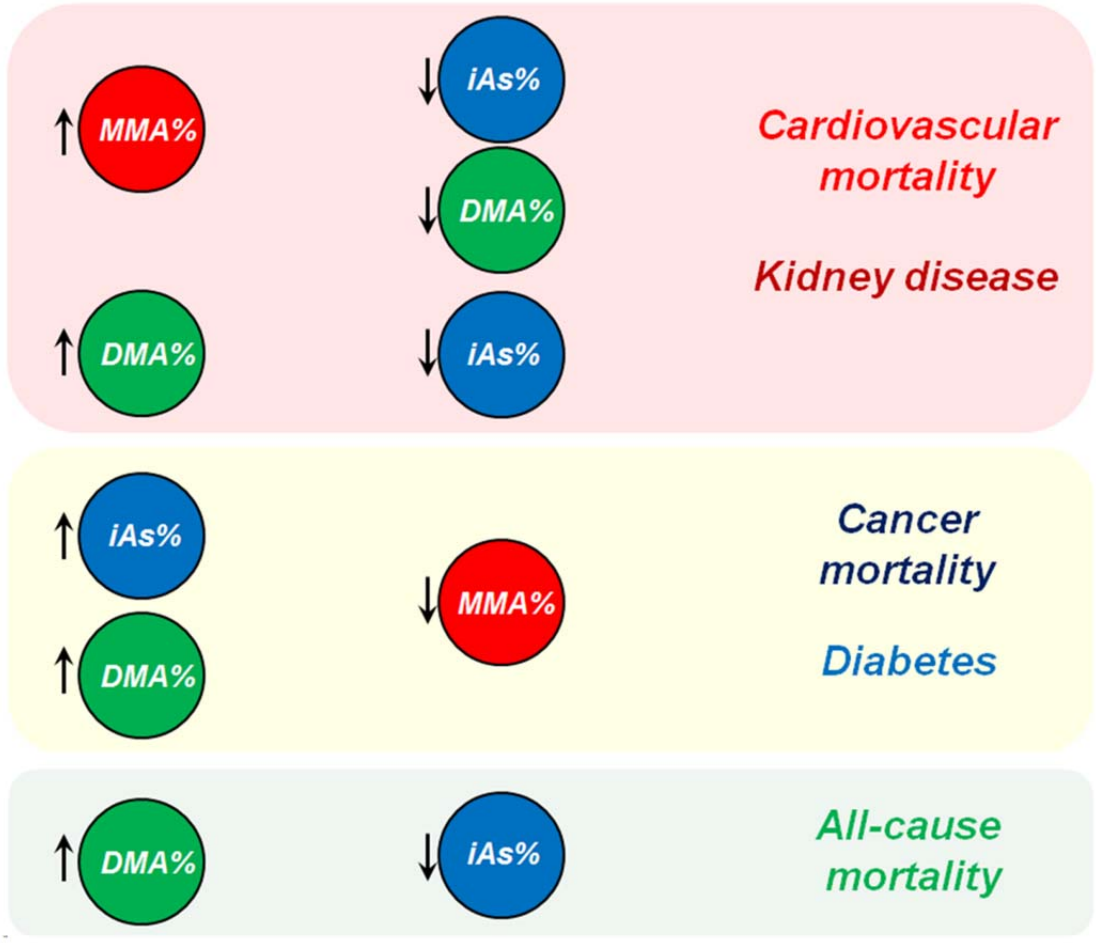
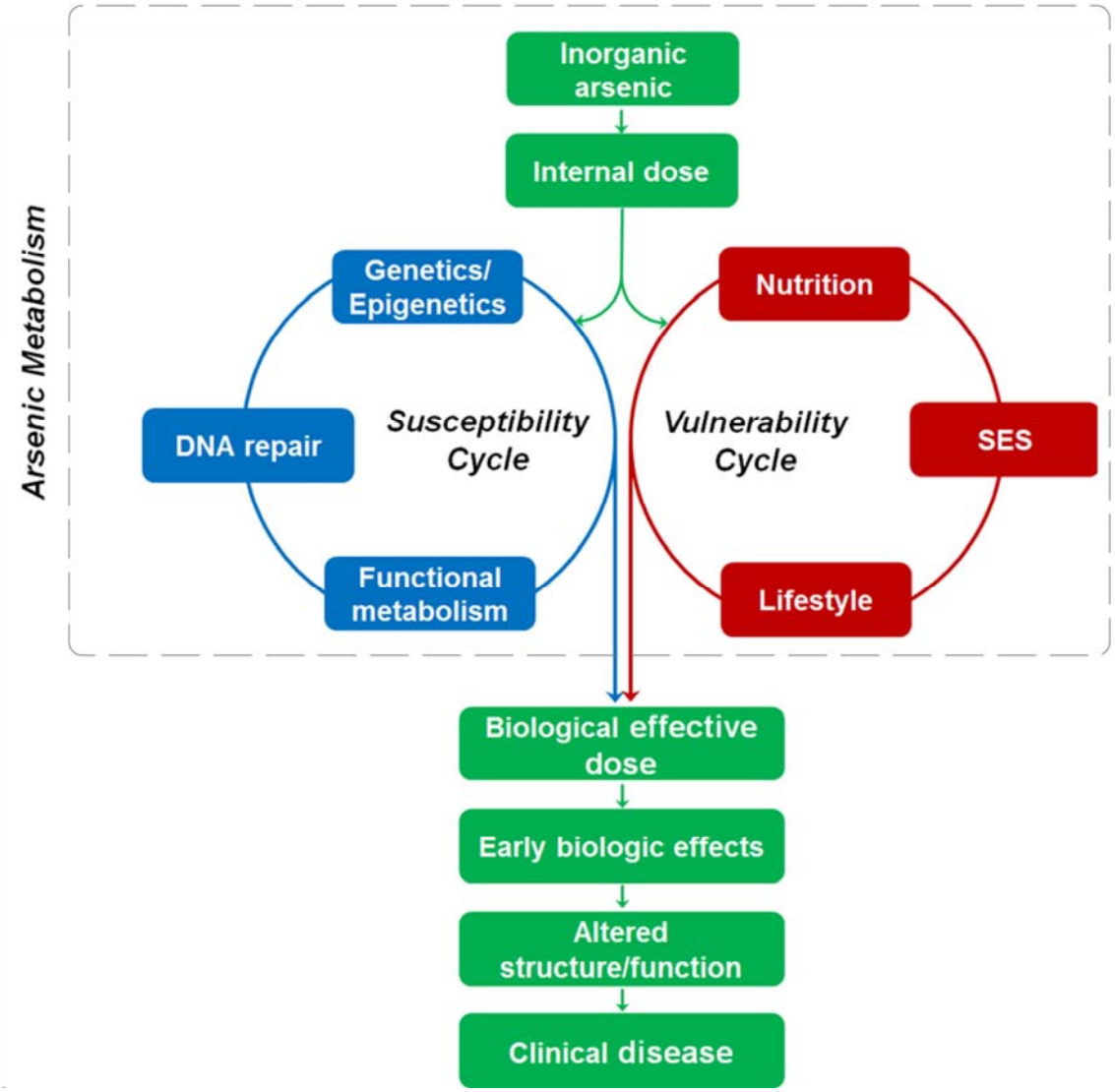


Figure 2. The role of arsenic metabolism in the classic toxicological paradigm. SES, socioeconomic status.



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EDUCATION and TRAINING

MD	1996-2003	China Medical University, Taichung, Taiwan
Intern	2002-2003	National Taiwan University Hospital, Taipei, Taiwan
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Fellow	2007-2009	Division of Nephrology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan
MPH	2011-2012	Johns Hopkins University Bloomberg School of Public Health, Maryland, USA
Language		Fluent in written and spoken English and Mandarin Chinese

RESEARCH FIELD

- Environmental risk factors and chronic diseases

PROFESSIONAL EXPERIENCE

Research & Teaching Assistant, Department of Epidemiology and Environmental Health Science, Johns Hopkins University, Maryland, USA (July 2012~)

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Attending Physician, Division of Nephrology, Department of Internal Medicine, National Taiwan University Hospital, Yun-Lin Branch, Yun-Lin County, Taiwan (July 2009 – Jun 2012)

HONORS AND AWARDS

•The Dyar Award, Department of Epidemiology, Johns Hopkins University, 2012-2013

•Taiwan Government Scholarship, 2011-2013

•Travel Grant Winner, World Congress of Nephrology (2011)

•Best Oral Presentation Paper, Annual Meeting of Taiwan Society of Nephrology 2009 (December 2009)

•Young Investigator Award, 7th Asian Pacific Congress of Hypertension (APCH) 2009 (February 2009)

•Top 10 BMJ Case Reports of 2009 (Rank 7/10) (February 2009)

•Best Resident Award, Department of Internal Medicine, National Taiwan University Hospital (2006)

CERTIFICATIONS

•Nephrology Certificate, Board of Taiwan Nephrology Society, Taiwan (August 2009)

•Internal Medicine Certificate, Board of Taiwan Society of Internal Medicine, Taiwan (September 2006)

•Medical License, National Medical Board, Taiwan (July 2003)

PUBLICATIONS

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