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<http://dx.doi.org/10.1289/ehp.1408742>

Received: 27 May 2014

Accepted: 10 June 2015

Advance Publication: 12 June 2015

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Running title: Arsenic exposure and cardiometabolic risk

Acknowledgments: This work was funded by NIH grants No. R01ES015326 and
3R01ES015326-03S1 to M.S. Support was also provided by the UNC Nutrition Obesity
Research Center grant No. DK056350 and by the Center for Environmental Health and
Susceptibility grant No. P30ES010126.

Competing financial interests: The authors declare that they do not have any competing
financial interests to disclose.

Abstract

Background: Exposure to arsenic (As) concentrations in drinking water $>150\mu\text{g/L}$ has been associated with risk of diabetes and cardiovascular disease, but less is known about effects of lower exposures. Few studies have examined whether moderate As exposure, or indicators of individual As metabolism at these levels of exposure, are associated with cardiometabolic risk.

Methods: We analysed cross-sectional associations between arsenic exposure and multiple markers of cardiometabolic risk using data from 1160 adults in Chihuahua, Mexico recruited in 2008-2013 with measures of drinking water As and urinary As species. Lipid and glucose levels in fasting blood, an oral glucose tolerance test, and blood pressure were used to characterize cardiometabolic risk. Multivariable logistic, multinomial and linear regression was used to assess associations between cardiometabolic outcomes and water As or the sum of inorganic and methylated As species in urine.

Results: After multivariable adjustment, concentrations in the second quartile of water As ($25.5\text{--}47.9\mu\text{g/L}$) and the $<$ median concentration of total speciated urinary As ($<55.8\mu\text{g/L}$) were significantly associated with elevated triglycerides, high total cholesterol, and diabetes. However, moderate water and urinary As levels were also positively associated with HDL cholesterol. Associations between arsenic exposure and both dysglycemia and triglyceridemia were higher among individuals with higher proportions of dimethyl-As in urine.

Conclusions: Moderate exposure to As may increase cardiometabolic risk, particularly in individuals with high proportions of urinary dimethyl-As. In this cohort, As exposure was associated with several markers of increased cardiometabolic risk (diabetes, triglyceridemia and cholesterolemia), but exposure was also associated with higher rather than lower HDL cholesterol.

Introduction

There is growing evidence that chronic exposure to inorganic As (iAs) may increase risk of cardiometabolic (CM) disorders, including diabetes mellitus (DM) and cardiovascular diseases (CVD) (Kuo et al. 2013; Maull et al. 2012; Moon et al. 2012). Experimental studies report adverse effects of iAs or its metabolites on mechanisms associated with CM disorders, such as insulin secretion and signaling, lipid metabolism, systemic inflammation, and atherosclerosis (Cheng et al. 2011; Douillet et al. 2013; Druwe et al. 2012; Fu et al. 2010; Lemaire et al. 2011; Muthumani and Prabu 2014; Paul et al. 2007). Recent reviews of the epidemiological literature suggest that exposure to levels of iAs in drinking water $>150\mu\text{g As/L}$ may increase risk of diabetes (Maull et al. 2012), and possibly CVD outcomes (Abhyankar et al. 2012; Moon et al. 2012; Navas-Acien et al. 2005). Evidence of relationships at low to moderate levels of exposure is more limited and equivocal.

Few epidemiologic studies to date have examined associations between moderate iAs exposure and markers of CM risk. Such studies may help to provide insights into the potential role of iAs exposure in the development and progression of CVD and diabetes. A few studies in industrially contaminated areas, or in settings with mean water As concentrations $>150\mu\text{g/L}$, have reported As exposure to be associated with CM markers such as elevated blood pressure, fasting glucose, triglycerides, and low-density lipoprotein cholesterol (LDL) (Chen et al. 2012; Karim et al. 2013; Wang et al. 2007). However, there is limited and inconsistent data on associations with CM risk markers, most notably dyslipidemias, at lower exposures (Abhyankar et al. 2012; Gribble et al. 2012; Jones et al. 2011).

Evidence is also limited on the role of iAs metabolism in determining health risks associated with iAs exposure. In humans, iAs is enzymatically methylated to yield methyl-As

(MAs) and subsequently dimethyl-As (DMAs) metabolites that are, along with residual iAs, excreted mainly in urine (Thomas et al. 2007). Urinary As profiles characterized by low percentages of DMAs and high percentages of MAs in urine are thought to indicate low capacity to methylate iAs. These indicators have been linked to an increased risk of cancer and precancerous skin lesions (Ahsan et al. 2007; Chen et al. 2003a; Chen et al. 2003b; Pierce et al. 2013; Yu et al. 2000). However, relationships between urinary profiles of iAs metabolites and non-cancerous outcomes remains unclear (Y. Chen et al. 2013b; Del Razo et al. 2011; Huang et al. 2007; Kim et al. 2013; Nizam et al. 2013).

This cross-sectional study explores associations between CM risk and chronic exposure to iAs in a recently established cohort of adult residents of Chihuahua (Mexico) who drink water with a wide range of iAs concentrations. We examine relationships between iAs in drinking water and urine, as well as urinary indicators of iAs metabolism, and CM risk based on measures of dysglycemia, including diabetes, dyslipidemia and blood pressure levels.

Materials and Methods

The Chihuahua cohort. All procedures involving human subjects were approved by IRBs in UNC Chapel Hill and Cinvestav-IPN (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City, Mexico). All participants provided signed informed consent. A total of 1160 adults (≥ 18 years old) with a minimum 5-year uninterrupted residency in the study area were recruited in household visits between 2008 and 2012. The participation rate was 67%. Other exclusions included pregnant women, persons with self-reported kidney or urinary tract infection (these conditions affect profiles of iAs metabolites in urine), and individuals with potential occupational exposure to As (e.g., those working with pesticides or in mines or smelters). Samples of drinking water were obtained from participants' households. An

interviewer-administered study questionnaire was used to record data on residency, occupation, drinking water sources and use, smoking, alcohol consumption, and medical history. As described previously (Currier et al. 2014), spot urines and fasting venous blood were collected during a morning medical exam which included an oral glucose tolerance test with blood drawn 2h after a 75g glucose dose. Plasma from both fasting and two-hour blood samples was stored at -80°C until analysis. Urine samples were aliquoted and immediately frozen. Trained staff obtained measures of weight without shoes and in light clothing to the nearest 0.1 kg, and height to the nearest 0.1 cm, used to calculate body mass index (BMI). BMI cutoffs of ≥ 25.0 , ≥ 30 , and $< 18.5 \text{ kg/m}^2$ were used to define overweight, obesity, and underweight individuals, respectively (WHO Expert Committee on Physical Status 1995). Waist circumference was measured at the midpoint between the lowest rib and the iliac crest. Blood pressure assessment used a manual sphygmomanometer. Three measures were taken at least one minute apart, with a 5 minute rest before the first reading; the mean of the last two measures was used. Participants were seated with their backs supported, feet on the floor, and the arm supported in the horizontal position, with the cuff at the level of the heart.

Arsenic analyses. Hydride generation-atomic absorption spectrometry coupled with a cryotrap (HG-CT-AAS) (Hernandez-Zavala et al. 2008) was used to determine the concentration of As in drinking water and concentrations of inorganic and methylated As species in urine. Arsenobetaine, arsenocholine and arsenosugars cannot not be measured by this method. A certified standard reference material, Arsenic Species in Frozen Human Urine (SRM 2669; National Institute of Standards and Technology <http://www.nist.gov/mml/csd/inorganic/arsenicurine.cfm>) was used to assure accuracy. Concentrations of As species measured by HG-CT-AAS in SRM 2669 ranged from 86.7 to

106.4% of the certified values. The limit of detection (LOD) for As in water as well as As species in urine was 0.01 μ gAs/L. Creatinine concentration in urine was determined by a colorimetric assay (Cayman Chemical Company, Ann Arbor, MI). Concentrations of water As and urinary As species which were below LOD (1.9% for water As, 1.6% for urinary iAs) were imputed at LOD/2. Total speciated As in urine (tAs) was calculated as sum of iAs, MAs and DMAs. The pattern of iAs metabolism was characterized using the percentage of tAs as DMAs, MAs and iAs, and the ratios of MAs/iAs and DMAs/MAs.

CM risk markers: A Prestige 24i Chemistry Analyzer was used to determine fasting plasma glucose (FPG) and 2h plasma glucose (2HPG) concentrations, and concentrations of triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL) in fasting plasma. Reference human sera (Serodos and Serodos PLUS, Human Diagnostics Worldwide) were used for quality control. LDL was calculated using the Friedewald equation, excluding 28 individuals with measured lipids outside accepted ranges for this approach (Oliveira et al. 2013). Diabetes was classified by FPG \geq 126mg/dL, 2HPG \geq 200mg/dL, or self-reported diabetes diagnosis or medication use (World Health Organization/International Diabetes Federation 2006). Pre-diabetes was defined as the absence of diabetes, with FPG \geq 110mg/dL or 2HPG \geq 140mg/dL. Individuals with diabetes or pre-diabetes were classified as having dysglycemia. Elevated fasting levels of each lipid were defined as plasma TG \geq 150mg/dL, TC \geq 200mg/dL, and LDL \geq 130mg/dL (Miller et al. 2011; NCEP 2002). Fasting HDL <40mg/dL in men and <50mg/dL in women were treated as low. Hypertension was defined by systolic blood pressure (SBP) >140mm Hg, diastolic blood pressure (DBP) >90mm Hg, or self-reported use of hypertensive medication (Chobanian et al. 2003).

Statistical analysis. Associations between iAs exposure and each CM risk marker were analyzed using both categorical and continuous exposure measures. Arsenic concentrations in water and urine, as well as urinary DMAs/MAs and MAs/iAs ratios, were either categorized in quartiles or natural log-transformed when used as continuous measures to improve their distributions. Associations with the percentage of urinary tAs comprised by DMAs, MAs and iAs are presented for quartiles or dichotomized at the median. Chi-square, ANOVA, and Kruskal-Wallis tests were used as appropriate to describe the significance of differences in subject characteristics by level of iAs exposure.

Multinomial (diabetes and pre-diabetes vs. neither) or simple logistic regression models (other variables) were used to analyze associations between iAs exposure and each CM risk outcome. To evaluate associations at various exposure doses, categorical as well as log-transformed continuous exposure variables were used. Models adjusted for age, gender, education, ethnicity, smoking, alcohol consumption, waist circumference, BMI, primary source of household drinking water (wells, treatment plants, and other), and self-reported seafood intake in the past week (a potential source of arsenobetaine or arsenosugars). Supplementary models examined the effect of adjusting for log-transformed urinary creatinine concentrations as recommended (Maull et al. 2012), or normalizing As concentrations by dividing by urinary creatinine. $P < 0.05$ was used to define statistical significance of main effects. Product terms (continuous outcomes) or relative excess risk for interaction (categorical outcomes) were calculated to assess interactions ($P < 0.10$) when exploring combined effects of iAs exposure and metabolism (Vanderweele and Knol 2014). Thus all interactions were evaluated on the additive scale. The primary analysis sample ($n=1090$, 94%) excluded individuals with missing data ($n=70$ for urinary tAs, blood pressure, dysglycemia, or covariates); 37 additional individuals were

missing lipid measures (n=1053). Water As measures were unavailable for an additional 52 participants in the analysis sample (N=1038; 1004 for lipids). Multiple imputations fit using 10 replicates of chained equations indicated that results of complete case analysis did not differ meaningfully when missing data were imputed (data not shown). All analyses used STATA version 13.1.

Results

As exposure: Sociodemographic and anthropometric characteristics of the Chihuahua cohort, as well as data characterizing CM risk prevalence, iAs exposure and urinary iAs metabolites are provided in Table 1. Concentrations of As in drinking water ranged from below detection to 419.8 μ g/L, with a median of 47.9 μ g/L. 83.3% of the analysis sample exceeded the US EPA and WHO recommended limit of 10 μ g As/L, and 75.3% exceeded the limit in Mexico of 25 μ g As/L (NOM-127-SSA 1994; US Environmental Protection Agency 2014). Concentration of total speciated urinary As (tAs) ranged from 0.52 to 491.5 μ g/L, with a median of 55.8 μ g/L. DMAs was the major metabolite (median 76.8% of tAs), followed by MAs (14.0%) and iAs (8.9%). Urinary tAs (Table 1) and concentrations of each As species increased with increasing concentrations of water As. However, the percentage of MAs and iAs increased with increasing amounts of urinary tAs (Supplemental Material, Table S1). Water As and urinary tAs were correlated (Spearman's rho=0.47).

Water As and CM risk. Overall, 18% of study participants had diabetes (115 of 183 reporting previous diagnosis), and 15% had pre-diabetes (Table 1). 41% had elevated TG, 23% high TC, 16% high LDL, and 42% had hypertension.

In multivariable-adjusted models (Table 2), drinking water As was associated with several markers of CM risk, including elevated TC and TG, as well as with diabetes (P<0.05 for

log water As), with increased risk in the second quartile ($\geq 25.5 \mu\text{g As/L}$), and no evidence of further increases in risk at higher exposures. However, greater exposure to water As was associated with reduced odds of low HDL, with patterns suggesting a monotonic dose-response ($P < 0.05$). Excluding individuals with diabetes ($N=183$) did not meaningfully affect relationships with other outcomes associated with water As [adjusted ORs (95% CI) for log-transformed water As were: 1.07 (95%CI: 1.01, 1.14) for TG, 1.07 (95%CI: 1.00, 1.15) for TC, and 0.87 (95%CI: 0.82, 0.93) for HDL].

Associations with continuous CM measures are shown in Supplemental Material, Table 2S. Although water As was not associated with pre-diabetes (Table 2), increasing exposure was associated with higher mean FPG and 2HPG among individuals not using diabetes medications, as well as among individuals without diabetes (Supplemental Material Table S2). After multivariable adjustment, water As was also associated with significant increases in mean FPG and 2HPG among fully normoglycemic participants (i.e., individuals without either diabetes or prediabetes; Figure 1). Consistent with categorical outcomes, after adjustment, water As was associated with increases in mean TG and TC and decreases in mean HDL, but not with mean LDL. Water As was not associated with mean DBP; an association with SBP was attenuated when individuals with diabetes were excluded (Supplemental Material Table S2).

Urinary tAs and CM risk. Urinary tAs concentrations were also associated with multiple markers of CM risk. As for water As, urinary tAs was associated with increased odds of diabetes and elevated TG, with evidence of increased risk at moderate concentrations (≥ 27.1 - $< 55.8 \mu\text{g/L}$) (Table 3). The highest quartile of tAs ($\geq 105 \mu\text{g/L}$) was associated with elevated TC ($P < 0.05$). There was, however, a reduced odds of low HDL associated with log tAs ($P < 0.05$). Additionally adjusting for urinary creatinine and urinary tAs metabolite composition (Table 3) tended to

strengthen the magnitude of associations. As with water As, urinary tAs was not associated with pre-diabetes, but was associated with significant increases in mean FPG among normoglycemic individuals in multivariable adjusted models (Figure 1); the highest quartile of tAs was also associated with mean increases in 2HPG. Excluding individuals with diagnosed diabetes did not meaningfully influence relationships between urinary tAs and other outcomes [adjusted ORs for the highest vs. lowest quartiles 1.71 (95% CI: 1.08, 2.71) for high TG; 2.14 (95% CI: 1.26, 3.62) for high TC; 0.71 (95% CI: 0.44, 1.12) for low HDL].

Relationships with continuous CM markers after multivariable adjustment (Supplemental Material Table S2) were similar to categorical outcomes, with urinary tAs positively associated with TG, TC, HDL, and FPG, as well as with 2HPG in subjects not using medications to control levels of those markers. Among individuals without diabetes, associations with 2HPG were attenuated. Urinary tAs was not associated with SBP or DBP even when normalized for creatinine [coefficients in individuals not using hypertensive medication 1.19 (95% CI: -0.29, 2.66) $P=0.12$ for SBP; 0.02 (95% CI: 0.88, 0.93) $P=0.95$ for DBP].

iAs metabolism and CM risk markers. Higher %DMAs and DMAs/MAs in urine were associated with increased odds of diabetes, elevated TG, and hypertension (Figure 2). Relationships between these indicators and other CM outcomes were non-linear and weak. Conversely, a higher %MAs was associated with reduced odds of diabetes, elevated TG, and hypertension. Like %MAs, a high MAs/iAs ratio was negatively associated with diabetes. Associations of this ratio with other outcomes did not reach significance ($P<0.05$), but in contrast to diabetes, generally suggested weak increases in risk. A high %iAs was associated with reduced odds of elevated TG [adjusted OR for the highest vs. lowest quartiles 0.58 (95% CI: 0.39, 1.86)]; other associations were weaker and non-significant.

iAs metabolism, iAs exposure and CM risk markers. We also examined joint effects of iAs metabolism and iAs exposure to assess whether associations between CM risk markers and As in drinking water varied depending on profiles of iAs metabolites in urine. For subjects with a high exposure to water As, odds of diabetes (Figure 3) as well as elevated TG (Supplemental Material, Table S3), were significantly increased when individuals had not only higher exposure, but also elevated %DMAs in urine (interaction $P < 0.10$). For example, the adjusted OR for diabetes associated with being in the highest vs. lowest quartile of water As was 2.61 (95% CI: 1.22, 5.57) for those with elevated %DMAs, but 0.87 (95% CI: 0.37, 2.04) for those with low DMAs. Similarly, an elevated %DMAs increased the odds of elevated TG associated with higher concentrations of urinary tAs (interaction $P < 0.10$; Table S3). The adjusted OR for the association between the highest vs. lowest quartiles of water As and high TG was 3.31 (95 %CI: 1.89, 5.78) vs. 1.18 (95% CI: 0.66, 2.08). Using continuous CM outcomes, among subjects without diabetes, the multivariable-adjusted mean increases in 2HPG and triglycerides associated with water As was significantly larger when %DMAs was elevated (interaction $P < 0.10$) (Table S2). Increases in mean FPG, 2HPG and triglycerides associated with higher urinary tAs were also stronger among individuals with elevated %DMAs (interaction $P < 0.10$ for all) (Supplemental Material Table S2).

Discussion

In this study, moderate exposure to As in drinking water, as well as modest concentrations of speciated urinary As, were associated with several CM risk markers. Water As concentrations $\geq 25.5 \mu\text{g/L}$ were associated with increased odds of diabetes, elevated plasma TG and TC. Similar concentrations of urinary tAs ($\geq 27.1 \mu\text{g/L}$) were also associated with diabetes and elevated TG, with higher levels ($\geq 105.0 \mu\text{g/L}$) associated with high TC. Though neither

water As nor urinary tAs were associated odds of with pre-diabetes (Tables 2 and 3), both were positively associated with increases in mean FPG and 2HPG among normoglycemic individuals (Figure 1). Unexpectedly, we found iAs exposure to be associated with decreased odds of low HDL. At the range of exposure in this study, associations with hypertension and blood pressure measures, or with LDL, were weak or null.

Using urinary As profiles to characterize capacity to metabolize iAs, we found that a higher %DMAs—or lower %MAs—was associated with increased odds of diabetes, elevated TG and hypertension. Moreover, the increased odds of diabetes and elevated TG associated with water As was stronger among individuals with an elevated %DMAs. This suggests that individuals with patterns of metabolism characterized by this marker may be more susceptible to adverse health outcomes associated with As exposure. Similarly, for urinary tAs, individuals with an elevated %DMAs had significantly higher mean increases in fasting and 2-hour glucose, and fasting TG and TC.

A higher %DMAs and lower %MAs, as well as higher DMAs/MAs and MAs/iAs ratios, have been proposed as indicators of more efficient enzymatic methylation of iAs (Del Razo 1997; Tseng 2007). Several studies in settings with high iAs exposure have reported a higher %MAs to be associated with markers of increased CM risk (Y. Chen et al. 2013a; Y. Chen et al. 2013b; Li et al. 2013a). Results from our independent population-based study in the Zimapan and Lagunera regions of Mexico suggest that the positive association with urinary DMAs species may be at least partly attributable to higher urinary concentrations of the toxic trivalent form of DMAs, DMAs^{III} (Del Razo et al. 2011). Notably, consistent with our findings, several recent studies also found a high DMAs/MAs ratio, a high %DMAs, or a lower %MAs in urine to be associated with increased risk of diabetes, metabolic syndrome, or individual CM risk markers

(Chen et al. 2012; Del Razo et al. 2011; Kim et al. 2013; Moon et al. 2013; Nizam et al. 2013).

Research is needed to assess the extent to which the toxic trivalent metabolites DMAs^{III} and MAs^{III} may influence CM risk associated with iAs exposure (Calatayud et al. 2014; Navas-Acien et al. 2006; Styblo et al. 2002).

In keeping with our findings, several recent prospective studies have found varying levels of iAs exposure—including median urinary As concentrations ≤ 20 $\mu\text{g/L}$ as well as >200 $\mu\text{g/L}$ —to be associated with increased morbidity and mortality from CVD outcomes including ischemic heart disease, stroke and diabetes (Chen et al. 2011; Y. Chen et al. 2013b; James et al. 2013; Kim et al. 2013; Moon et al. 2012; Moon et al. 2013; Navas-Acien et al. 2008, 2009). Earlier cross-sectional studies in settings with high levels of As exposure reported water or hair arsenic to be associated not only with elevated TC, TG and fasting glucose, but also with higher LDL, lower HDL, elevations in both SBP and DBP, and systemic inflammation (Chen et al. 2012; Karim et al. 2013; Wang et al. 2007). These studies suggest the possibility of additional or more severe CM effects at higher levels of exposure.

This study found a high proportion of DMAs to be associated with hypertension. However, associations between iAs exposure and hypertension were weak and non-significant, perhaps in part due to the moderate exposure levels. Notably, associations with hypertension have been heterogeneous in areas with water As below 400 $\mu\text{g/L}$, albeit consistent at higher exposures (Abhyankar et al. 2012; Islam et al. 2012; Jones et al. 2011). Normalizing vs. adjusting for creatinine slightly strengthened associations with urinary As; however studies reporting associations with hypertension using this metric (Li et al. 2013b) have also had considerably higher exposures than in this population: median 136 μg vs. 47.4 μg As/g creatinine. Future research should assess whether moderate exposure may be more closely linked to

alternative indicators of vascular function (Kunrath et al. 2013; Li et al. 2013b; Wu et al. 2012), or may vary in genetically or nutritionally vulnerable subgroups with varying iAs metabolism (Chen et al. 2007).

Despite increases in mean 2HPG and FPG associated with iAs exposure among normoglycemic individuals (Figure 1), iAs exposure was not associated with pre-diabetes. It may be that the cutoffs used to characterize pre-diabetes are not sufficiently sensitive in our population, that iAs exposure may promote rapid progression to more severe disease, or that association with glucose measures are not causal.

Though further prospective studies are needed to evaluate these relationships, laboratory studies suggest that iAs or its metabolites may inhibit insulin secretion or signaling (Douillet et al. 2013; Fu et al. 2010; Paul et al. 2007), alter lipid metabolism (Cheng et al. 2011; Hossain et al. 2013; Muthumani and Prabu 2014), and generate pro-inflammatory responses (Calatayud et al. 2014; Druwe et al. 2012). Increases in TC, LDL and TG—along with decreases in HDL—have been observed in rodents treated with iAs (Muthumani and Prabu 2014), along with increases in hypertension, cardiac hypertrophy and atherosclerosis (Cheng et al. 2011; Lemaire et al. 2011; Sanchez-Soria et al. 2012). The unexpected association with HDL requires further study in diverse populations and in laboratory settings. It is important to note that large disparities in the prevalence, predictors and health consequences of low HDL have been described in Mexican and other Hispanic populations compared to those of European descent (Aguilar-Salinas et al. 2001; Morales et al. 2014; Paramsothy et al. 2010; Salas et al. 2014).

Despite the limitation of a cross-sectional design, findings from this study are largely consistent with experimental research and several smaller epidemiological studies in high exposure settings. Moreover, although personal exposure was characterized based on a single

urine sample, findings for water As and urinary tAs were largely consistent. Few previous studies provide comparisons of urinary and water As measures, or use urinary indicators to assess how metabolism may modify health effects of environmental exposure through water (Y. Chen et al. 2013a; Y. Chen et al. 2013b). The consistency of unadjusted and multivariable-adjusted results, along with those of sensitivity analyses excluding individuals previously diagnosed with hypertension or diabetes who may have adjusted behaviors such as water consumption, also supports the possibility of a causal relationship. Moreover, the high prevalence of obesity and cardiometabolic risk closely resembles those in the general population reported for Mexico (Barquera et al. 2009; Salas et al. 2014; Villalpando et al. 2010) does not suggest selectivity in our cohort with respect to these outcomes. Studies in such settings may help to provide a more complete understanding of how iAs exposure may influence cardiometabolic risk, as many previous studies on these relationships have been conducted in settings such as Bangladesh, where the prevalence of obesity and CM disorders is relatively low (Yu Chen et al. 2013; Pan et al. 2013).

In summary, results of this study suggests potential CM risks associated with chronic exposure to As at levels below 100 μ g/L in drinking water, filling a current gap in knowledge (Maull et al. 2012; Moon et al. 2013). Associations with measures of dyslipidemia, which have been less studied to date, warrant further study, given that the implications of our results for health risks were inconsistent for HDL, LDL and triglycerides. Studies that incorporate measures of specific lipid fractions and particles may be better able to evaluate the health risks of any association with iAs exposure (Genest 2008; Vickers and Remaley 2014). Findings also suggest that iAs metabolism may influence the extent to which environmental exposure to iAs adversely affects risk of CM impairment. Studies which measure trivalent as well as pentavalent urinary As

species are needed to better understand the impact of metabolism on health risks associated with iAs exposure.

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Table 1. Characteristics of the sample by concentration of arsenic in household water. Data are N (%), mean \pm SD, or median (25th–75th percentile) unless otherwise indicated.

Characteristic	All participants	Household water arsenic ($\mu\text{g/L}$) quartiles			
		<25.5	≥ 25.5 -<47.9	≥ 47.9 -<79.0	≥ 79.0
Total N	1038	260	260	259	259
<i>Sociodemographic, lifestyle</i>					
Age, y*	45.6 \pm 15.9	47.4 \pm 16.8	43.4 \pm 16.4	44.6 \pm 14.5	47.0 \pm 15.4
Female	712 (68.6)	180 (69.2)	174 (66.9)	185 (71.4)	173 (66.8)
Higher than primary education*	320 (30.8)	93 (35.8)	106 (40.8)	71 (27.4)	50 (19.3)
Smokes	291 (28.0)	65 (25.0)	71 (27.6)	70 (27.0)	85 (32.8)
Drinks alcohol**	423 (40.8)	90 (34.6)	114 (43.9)	115 (44.4)	104 (40.2)
Recent seafood**	260 (25.1)	79 (30.4)	64 (24.6)	64 (24.7)	53 (20.5)
<i>Anthropometric^a, cardiometabolic</i>					
Weight status ^{a**}					
Overweight	368 (35.5)	91 (35.0)	88 (33.9)	87 (33.6)	102 (39.4)
Obese	411 (39.6)	92 (35.4)	112 (43.1)	118 (45.6)	89 (34.4)
Waist circumference, cm					
Females**	98.8 (13.0)	96.2 (12.0)	100.7 (12.9)	100.1 (14.5)	98.2 (12.1)
Males	96.7 (12.1)	97.7 (11.8)	96.5 (12.0)	97.0 (12.3)	95.6 (12.2)
Dysglycemia ^b					
Diabetes	183 (17.6)	33 (12.7)	53 (20.4)	47 (18.2)	50 (19.3)
Pre-diabetes	156 (15.0)	41 (15.8)	37 (14.2)	38 (14.7)	40 (15.4)
Triglycerides ≥ 150 mg/dL*	412 (41.0)	85 (33.5)	104 (41.4)	110 (43.8)	113 (45.6)
Total cholesterol ≥ 200 mg/dL**	234 (23.3)	44 (17.3)	61 (24.3)	67 (26.7)	62 (25.0)
LDL cholesterol ≥ 130 mg/dL ^c	160 (16.3)	33 (13.2)	43 (17.4)	45 (18.5)	39 (16.3)
HDL < 40/50 mg/dL**	589 (58.7)	161 (63.4)	151 (60.1)	144 (57.4)	133 (53.6)
Hypertension ^d	439 (42.3)	106 (40.8)	106 (40.8)	109 (42.1)	118 (45.6)
<i>Urinary As and dilution markers</i>					
Total As ^e ($\mu\text{g/L}$)*	55.8 (27.1-105)	22.9 (6.5-48.3)	59.0 (35.4-94.3)	62.6 (33.4-101)	96.6 (52.0-150)
DMAs ($\mu\text{g/L}$)*	42.4 (20.5-77.6)	16.2 (5.1-35.0)	44.0 (26.3-71.7)	62.6 (33.4-101)	96.6 (52.0-150)
MAs ($\mu\text{g/L}$)*	7.7 (3.2-14.9)	2.7 (0.8-7.3)	8.3 (4.5-13.5)	47.5 (25.9-77.5)	69.5 (38.5-115)
iAs ($\mu\text{g/L}$)*	5.0 (1.9-10.0)	1.5 (0.5-4.8)	5.7 (2.7-9.3)	8.7 (4.4-14.4)	13.5 (6.0-24.0)
DMAs/MAs	5.5 (4.0-7.4)	5.6 (4.1-7.5)	5.7 (4.2-7.6)	5.4 (4.1-7.4)	5.3 (3.8-7.1)
MAs/iAs	1.6 (1.2-2.1)	1.6 (1.1-2.3)	1.5 (1.1-2.0)	1.6 (1.2-2.1)	1.6 (1.2-2.0)
% DMAs	76.8 (70.6-81.5)	76.7 (70.3-81.0)	76.6 (71.3-81.3)	77.2 (71.6-82.8)	76.6 (69.6-81.2)
% MAs	14.0 (10.9-17.7)	13.9 (10.8-17.3)	13.5 (10.5-13.7)	14.2 (11.1-17.7)	14.4 (11.2-18.4)
% iAs	8.9 (6.4-12.3)	8.8 (6.0-12.9)	9.4 (6.4-12.8)	8.5 (6.4-11.6)	8.9 (6.7-12.1)
Creatinine, mg/dL*	135 (74.7-173)	115 (60-162)	131 (78-190)	140 (80-183)	144 (82-167)

One-way anova, Pearson's chi-square or Kruskal-Wallis test for differences across increasing quartiles of water As * P<0.05 ** P<0.10. Distributions among individuals with household water As, N=1038 for all variables except LDL (N=980) and other lipids (N=1004).

^aWeight status: BMI ≥ 25 -<30 overweight, BMI ≥ 30 obese. ^bDiabetes: fasting plasma glucose (FPG) ≥ 126 mg/dL, 2h plasma glucose (2HPG) ≥ 200 mg/dL, or self-reported diabetes diagnosis or medication use. *Pre-diabetes*: FPG ≥ 110 - <126 mg/dL or 2HPG ≥ 140 mg/dL. ^cLDL-cholesterol: estimated using the Friedewald equation if triglycerides <400 mg/dL (Oliveira et al. 2013). ^dHypertension: SBP>140mm Hg, DBP>90mm Hg or hypertensive medication use [medication use reported by n=126 (28.7%) of the hypertensive individuals]. ^eTotal speciated urinary arsenic: Σ [dimethylated (DMAs), mono-methylated (Mas) and inorganic (iAs) arsenic species].

Table 2. Household drinking water arsenic concentrations and prevalent cardiometabolic risk outcome: Odds ratios (95% CI).

Cardiometabolic outcome	Water arsenic exposure quartile (µg/L)			Ln-Water As (µg/L)
	≥25.5-<47.9 µg/L	≥47.9-<79.0 µg/L	≥79.0 µg/L	
Dysglycemia ^a				
Diabetes	2.46 (1.44, 4.21)*	1.74 (1.01, 2.99)*	1.65 (0.97, 2.81)**	1.14 (1.05, 1.25)*
Prediabetes	1.14 (0.68, 1.91)	1.04 (0.62, 1.73)	1.13 (0.68, 1.88)	1.00 (0.94, 1.09)
Triglycerides ≥150 mg/dL	1.45 (0.99, 2.14)**	1.53 (1.04, 2.24)*	1.69 (1.15, 2.49)*	1.09 (1.03, 1.15)*
Total Cholesterol ≥200 mg/dL	1.75 (1.11, 2.74)*	1.89 (1.21, 2.95)*	1.65 (1.05, 2.59)*	1.08 (1.01, 1.16)*
LDL ≥130 mg/dL ^b	1.54 (0.92, 2.56)**	1.59 (0.96, 2.65)**	1.35 (0.80, 2.27)	1.04 (0.96, 1.12)
HDL <40/50 mg/dL	0.78 (0.52, 1.17)	0.63 (0.42, 0.93)*	0.59 (0.40, 0.88)*	0.87 (0.82, 0.93)*
Hypertension ^c	1.30 (0.84, 2.00)	1.27 (0.82, 1.94)	1.41 (0.91, 2.17)	1.03 (0.97, 1.10)

*P<0.05 **P<0.10 for odds ratios for elevated vs. low cardiometabolic risk associated with increasing water As exposure vs. the lowest quartile (<25.5µg/L). Results come from multinomial or logistic models adjusted for age, gender, education, smoking status, alcohol consumer, recent seafood intake, weight status, elevated waist circumference and main water source (well, treatment plant, or other); multinomial models used for diabetes and pre-diabetes vs. neither; logistic models used for other outcomes.

^a*Diabetes*: fasting plasma glucose (FPG) ≥126 mg/dL, 2h plasma glucose (2HPG) ≥200 mg/dL, or self-reported diabetes diagnosis or medication use. *Pre-diabetes*: FPG ≥110 -<126 mg/dL or 2HPG ≥140 mg/dL. Normoglycemic individuals (i.e. individuals with no diabetes or pre-diabetes) are the referent. ^b*LDL-cholesterol*: estimated using the Friedewald equation if triglycerides<400 mg/dL (Oliveira et al. 2013). ^c*Hypertension*: SBP>140, DBP>90 or use of hypertensive medication [medication use reported by 27.9% of hypertensive individuals]. Normotensive individuals (no stage 1 or 2 hypertension) are the referent.

Table 3. Total speciated urinary As and prevalent cardiometabolic risk: Odds ratios (95% CI).

Cardiometabolic outcome	Total urinary speciated arsenic quartiles, µg/L			Ln-Total Urinary As (µg/L)
	≥27.1-<55.8	≥55.8-105.0	≥105.0	
N	272	273	272	--
<i>Multivariable adjusted</i>				
Dysglycemia ^a				
Diabetes	1.57 (0.94-2.63)**	1.56 (0.92-2.65)**	1.99 (1.19-3.33)*	1.29 (1.09-1.53)*
Prediabetes	0.92 (0.56-1.53)	1.21 (0.74-1.98)	1.15 (0.69-1.92)	1.04 (0.89-1.23)
Triglyc. ≥150mg/dL	1.39 (0.95 - 2.02)**	1.47 (1.01 - 2.13)*	1.80 (1.23 - 2.64)*	1.23 (1.08-1.39)*
Choles. ≥200 mg/dL	1.15 (0.74-1.78)	1.35 (0.88-2.07)	1.54 (1.00-2.38)*	1.15 (1.00-1.33)*
LDL ≥130 mg/dL ^{2b}	0.99 (0.60-1.62)	1.22 (0.75-1.99)	1.25 (0.76-2.05)	1.09 (0.93-1.28)
HDL <40/50 mg/dL	0.94 (0.64-1.27)	1.09 (0.74-1.60)	0.82 (0.56-1.21)	0.82 (0.72-0.93)*
Hypertension ³	0.67 (0.44-1.01)*	0.60 (0.40-0.92)*	0.77 (0.50-1.17)	0.93 (0.72-1.07)
<i>Additionally adjusted for creatinine and elevated % DMAs, MAs, iAs in urine</i>				
Dysglycemia				
Diabetes	1.76 (1.03-3.02)*	1.98 (1.12-3.50)*	2.78 (1.55-5.00)*	1.45 (1.19-1.77)*
Prediabetes	0.89 (0.53-1.51)	1.22 (0.72-2.08)	1.16 (0.65-2.04)	1.04 (0.86-1.25)
Triglyc. ≥150mg/dL	1.41 (0.95 - 2.08)**	1.55 (1.04-2.32)*	1.96 (1.28 - 3.00)*	1.25 (1.08-1.44)*
Choles. ≥200 mg/dL	1.25 (0.80-1.96)	1.56 (0.98-2.47)**	1.89 (1.16-3.06)*	1.22 (1.04-1.44)*
LDL ≥130 mg/dL	1.08 (0.65-1.81)	1.42 (0.84-2.40)	1.54 (0.88-2.69)	1.16 (0.96-1.40)
HDL <40/50 mg/dL	0.92 (0.62-1.36)	1.09 (0.73-1.64)	0.81 (0.52-1.25)	0.87 (0.77-0.99)*
Hypertension ^c	0.74 (0.48-1.14)	0.73 (0.47-1.14)	1.02 (0.64-1.61)	1.03 (0.89-1.20)

*P<0.05 **P<0.10 for odds ratios for elevated vs. low cardiometabolic risk associated with increasing water As exposure vs. referent of <27.5µg/L (n=273). Results come from multinomial or logistic models adjusted for age, gender, education, smoking status, alcohol consumer, recent seafood intake, weight status, elevated waist circumference and main water source (well, treatment plant, or other); multinomial model used for diabetes and pre-diabetes vs. neither; logistic models used for other outcomes. Ln-transformed urinary creatinine and > median % DMAs, Mas, and iAs in urine additionally included in models as indicated.

^a*Diabetes*: fasting plasma glucose (FPG) ≥ 126 mg/dL, 2h plasma glucose (2HPG) ≥ 200 mg/dL, or self-reported diabetes diagnosis or medication use. *Pre-diabetes*: FPG ≥ 110 - < 126 mg/dL or 2HPG ≥ 140 mg/dL. Normoglycemic individuals (i.e. individuals with no diabetes or pre-diabetes) are the referent. ^b*LDL-cholesterol*: estimated using the Friedewald equation if triglycerides < 400 mg/dL (Oliveira et al. 2013). ^c*Hypertension*: SBP > 140 , DBP > 90 or use of hypertensive medication [medication use reported by 27.9% of hypertensive individuals]. Normotensive individuals (no stage 1 or 2 hypertension) are the referent.

Figure Legends

Figure 1. Adjusted mean (95% CI) difference in fasting or 2h plasma glucose associated with As exposure among normoglycemic subjects. Adjusted mean (95% CI) difference in glucose measure for increasing quartiles of water As or total speciated urinary As, relative to individuals in the lowest quartile (<25.5µg/L for water and <27.1µg/L for urine). Estimated from linear regression models including age, gender, education, ethnicity, weight status, waist circumference, smoking status, alcohol consumption, recent seafood intake, and water source (well, treatment plant, or other). Urinary As models additionally adjust for urinary creatinine and \geq median %DMAs, MAs and iAs. Models excluded individuals with 2h plasma glucose >140mg/dL, fasting plasma glucose >110 mg/dL, or diagnosed diabetes. Among normoglycemic subjects: N in each quartile of water As 1=186, 2=170, 3=174, 4=169; of urinary As: 1=195, 2=181, 3=186, 4=170.

Figure 2. Associations between urinary As metabolism indicators and cardiometabolic risk.

* P<0.05. Odds ratios (95% CIs) for elevated cardiometabolic risk associated with increasing quartiles of urinary iAs metabolism indicators from multinomial or logistic models adjusted for total speciated urinary arsenic, as well as age, gender, education, ethnicity, weight status, waist circumference, smoking status, alcohol consumption, recent seafood intake, and water source (well, treatment plant, or other). N=1090 adults.

-Cardiometabolic markers: DM=diabetes mellitus, TG=triglycerides, TC=total cholesterol, LDL=low density lipoprotein cholesterol, HDL=high density lipoprotein cholesterol, HTN=hypertension.

-Urinary As indicators: DMAs=dimethyl-As; MAs=methyl-As; iAs=inorganic As.

-Cardiometabolic outcomes defined as: DM=fasting plasma glucose \geq 126 mg/dL, 2h plasma glucose \geq 200 mg/dL, or self-reported diabetes diagnosis or medication use; elevated TC \geq 200 mg/dL; elevated TG \geq 200 mg/dL; elevated LDL \geq 130 mg/dL; low HDL= $<$ 40 mg/dL; hypertension SBP >140 mm Hg, DBP>90 or hypertensive medication use.

-Quartile markers (1st=referent): 2nd=black diamond, 3rd textured square, 4th gray circle.

-Quartile cutoffs for urinary As metabolism indicators defined as: %DMAs = <70.65, 70.65-<76.78, 76.78-<81.52, \geq 81.52; %MAs = <10.90, 10.90-<14.0, 14.0-<17.66, \geq 17.66;

DMAs/MAs = <4.05, 4.05-<5.47, 5.49-<7.38, \geq 7.38; MAs/iAs = <1.185, 1.185-<1.576, 1.576-<2.11, \geq 2.11.

Figure 3. Water As and odds of prevalent diabetes in subjects with vs. without elevated % urinary DMAs. * P<0.10 for additive interaction (relative excess risk for interaction) for the joint effect of water As and high %DMAs. ORs (95% CI) for prevalent diabetes associated with household water As categories (<25, 25-50, 50-100 and \geq 100 μ g/L), in subjects with proportions of DMAs defined as low vs high based on the median of 76.6%. The referent group for all ORs is subjects with %DMAs below the median in the lowest quartile of water As. Results are from multinomial models adjusted for age, gender, smoking status, alcohol consumer, BMI, elevated waist circumference and main water source (well, treatment plant, or other

Figure 1. Adjusted mean (95% CI) difference in fasting or 2h plasma glucose associated with As exposure among normoglycemic subjects

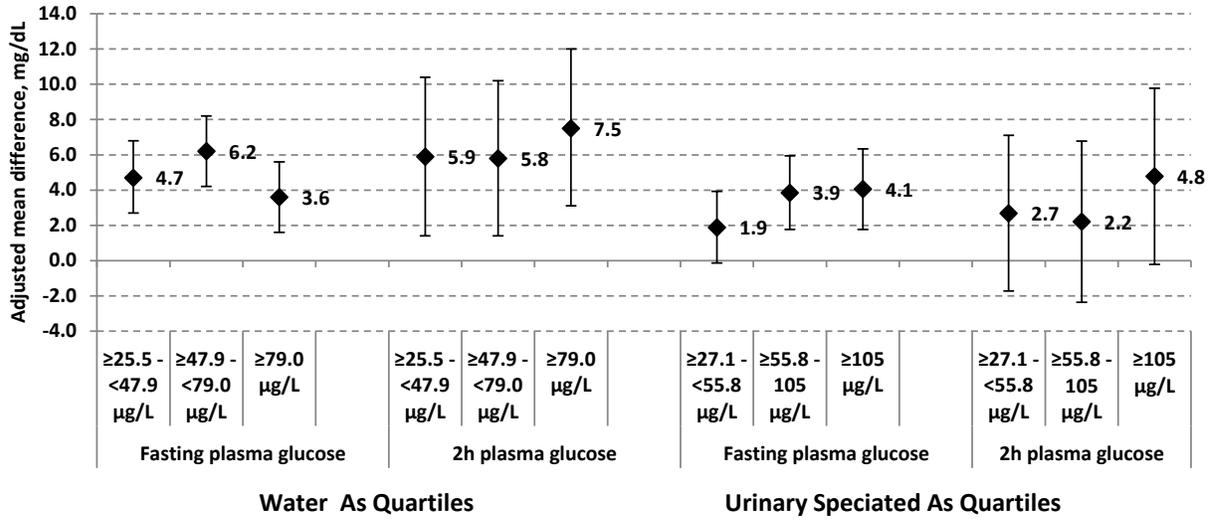


Figure 2. Associations between urinary As metabolism indicators and cardiometabolic risk

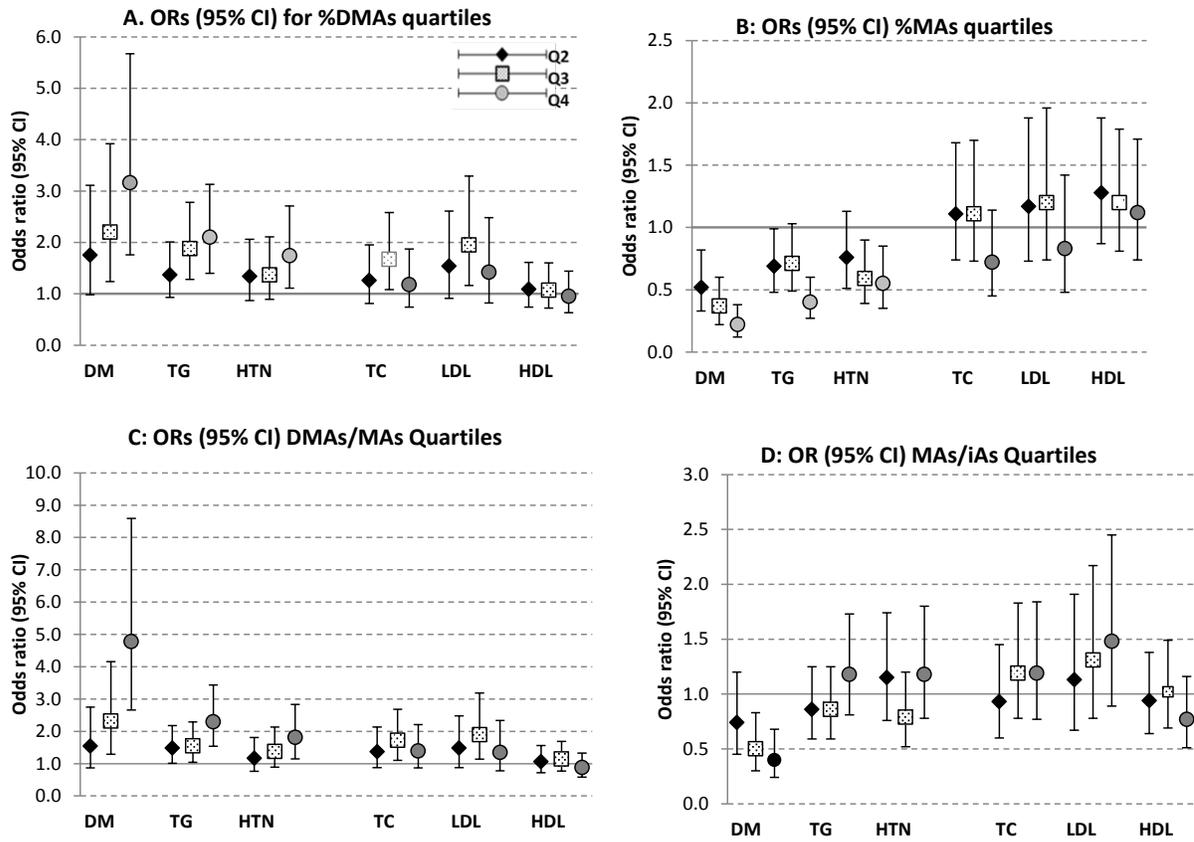


Figure 2 continued: panel E

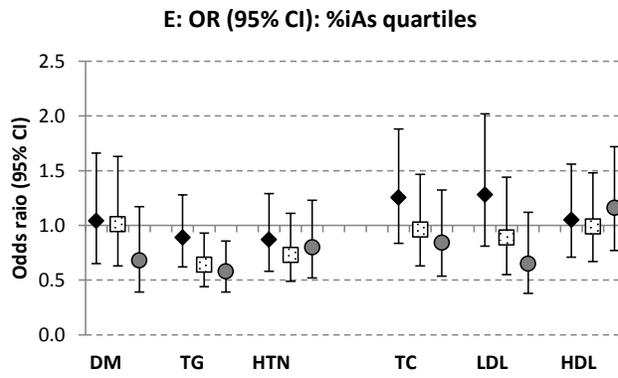


Figure 3. Water As and odds of prevalent diabetes in subjects with vs. without elevated % urinary DMAs

