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Urinary Arsenic and Insulin Resistance in US Adolescents

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Abstract

Chronic arsenic exposure has been associated with increased diabetes risk in adults. Insulin resistance (IR) has been proposed as a mechanism of arsenic-related diabetes. Although limited evidence in adults found no association between arsenic and IR, the association in adolescents is largely unknown. We examined the association between urinary arsenic and insulin resistance in US adolescents. Eight hundred thirty five adolescents aged 12-19 years, with complete data on urinary arsenic (total arsenic, inorganic arsenic and dimethylarsenic acid (DMA)), fasting glucose, insulin and key covariates were identified from the National Health and Nutrition Examination Survey (NHANES) cycles 2003/2004 through 2009/2010. Generalized additive mixed models accounting for intra-cluster correlation arising from the complex survey design were used to estimate the association between the updated Homeostasis Model Assessment (HOMA2)-IR and each type of arsenic. After adjusting for potential confounders, including urinary creatinine, sociodemographic factors, BMI, waist circumference, and arsenobetaine, arsenic exposure was not associated with HOMA2-IR. Interquartile range increases in total arsenic, inorganic arsenic and DMA were associated with 1.5% (95% CI: -2.0, 5.2), 1.1% (95% CI: -1.5, 3.8) and 0.25% (95% CI: -2.3, 2.9) increases in HOMA2-IR, respectively. In conclusion, despite arsenic's association with diabetes in adults and potential role in insulin resistance, our findings do not support the hypothesis that arsenic exposure at levels common in the US contributes to insulin resistance in adolescents. Whether higher doses and longer exposure duration are required for appreciable influence on insulin resistance, or that arsenic does not act through insulin resistance to induce diabetes needs further investigation.[1]

[1]**Abbreviations:** iAs-inorganic arsenic; DMA-dimethylarsenic acid; IR-insulin resistance; HOMA2-the updated Homeostasis Model Assessment; HOMA2-IR-insulin resistance index HOMA2 calculates; PIR- ratio of family income to poverty

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Keywords

Arsenic; Insulin resistance; Adolescents; NHANES

Introduction

Approximately thirteen million Americans live in areas with elevated inorganic arsenic (>10 μg/L) in water supplies (EPA, 2001); many of them are adolescents. Besides water, rice, certain fruit juices and seafood also contribute to arsenic exposure. Indeed, a recent study revealed that arsenic was detectable in the urine of 99.4% of Americans between 6 and 17 years old, with especially high levels among rice-eaters (Davis et al., 2012). Such widespread exposure underscores the importance of understanding arsenic's health effects in adolescents, even at the relatively low exposure levels common in the United States.

One of the health effects associated with arsenic is diabetes (mainly type-2) in adults. Over the past decades, studies in diverse regions worldwide have reported that chronic exposure to inorganic arsenic at even low to moderate levels may increase the risk of diabetes in adults (Coronado-González et al., 2007; Gribble et al., 2012; Navas-Acien et al., 2008; Tseng et al., 2000). Moreover, *in vitro* studies have shown that arsenic treatment could disrupt a number of biochemical processes involved in glucose homeostasis, leading to both decreased insulin-stimulated glucose uptake and decreased glucose-stimulated insulin secretion (Fu et al., 2010; Maull et al., 2012; Paul et al., 2007). Consequently, arsenic is thought to induce diabetes in at least two ways. First, chronic arsenic exposure may reduce insulin-stimulated glucose uptake in muscles and fat tissues, leading to insulin resistance, which then contributes to diabetes. Second, chronic exposure may damage pancreatic β cells, resulting in impaired insulin secretion, which eventually manifests as diabetes (Tseng, 2004).

We are interested in the insulin resistance pathway among adolescents, because insulin resistance is known to occur in this age group and is an important predictor of type-2 diabetes in adulthood (Steinberger, 2003). Additionally, air pollution and phthalates have been associated with insulin resistance in children, indicating possible environmental origins of the condition (Thiering et al., 2013; Trasande et al., 2013). Lastly, although the few studies in adults did not find positive associations between arsenic exposure and insulin resistance, they are limited to middle-aged populations in which diabetes is already prevalent (Del Razo et al., 2011; Gribble et al., 2012; Rhee et al., 2013). If arsenic indeed leads to diabetes through insulin resistance, we may be able to detect an association among younger populations but not in middle-aged adults because those who survive to middle age diabetes-free may be intrinsically resistant to arsenic's effects. A recent study among Taiwanese children seems to support this idea, but exposure levels in that study were relatively high (Lin et al., 2014). Whether arsenic exposure at levels common in the US increases insulin resistance in adolescents is unknown. For these reasons, we examined the association between urinary arsenic and insulin resistance, measured by the updated Homeostasis Model Assessment (HOMA2), among a sample of adolescents from the general U.S. population.

Materials and methods

Study population

The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional survey with a complex, multi-stage, probability sampling design. In NHANES cycles 2003/2004 through 2009/2010, 18,983 participants were between 12-19 years old. After excluding 105 pregnant girls, 1,130 individuals were identified to have been selected for both urinary arsenic analysis and fasting glucose and insulin measurements. Of these, 45 and 104 were excluded due to missing urinary arsenic and fasting glucose/insulin measures, respectively. Additionally, 63 adolescents who fasted for less than eight hours or whose hours of fasting were unknown; 3 who were currently using insulin or oral hypoglycemic agents; 1 whose fasting glucose and insulin values were clinically unrealistic; and 79 who had missing data in core covariates (serum cotinine, urinary creatinine, body mass index (BMI), waist circumference or poverty income ratio (PIR)) were excluded, leaving a final analytical sample of 835 individuals. Compared with excluded individuals, included individuals had significantly lower fasting insulin levels (percent difference (included vs. excluded) = -18.0%, 95% CI: -26.2, -8.79-, p=0.0003), lower fasting glucose levels (difference in mg/dL (included vs. excluded)= -3.5, 95% CI: -6.1, -0.94, p-value=0.008) and were slightly younger (age difference in year (included vs. excluded)= -0.39, 95% CI: -0.69, -0.088, p-value=0.01).

All participants of NHANES provided written informed consent consistent with requirements of the National Center for Health Statistics Institutional Review Board. NHANES data are publicly available.

Urinary total arsenic and speciated arsenics

Participants were asked to provide a spot urine sample at the mobile examination center (MEC). Urinary total arsenic was analyzed with inductively coupled-plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS) on an ELAN® DRC^{Plus} or an ELAN® DRC™ II ICP-MS (PerkinElmer SCIEX, Concord, ON, Canada). Arsenic species, including arsenous acid, arsenic acid, arsenobetaine, arsenocholine, dimethylarsinic acid (DMA), monomethylarsonic acid and trimethylarine oxide, were analyzed with high performance liquid chromatography (HPLC) coupled to ICP-DRC-MS (National Center for Environmental Health, 2004). The limit of detection (LOD) of total arsenic was 0.6μg/L in cycle 2003/2004 and 0.74 μg/L from 2005 through 2010. The LOD of arsenic species were constant between 2003 and 2010 (arsonous acid: 1.2μg/L, arsenic acid: 1.0μg/L, arsenobetaine: 0.4 μg/L, arsenocholine: 0.6 μg/L, DMA: 1.7 μg/L, monomethylarsonic acid: 0.9 μg/L, trimethylarsine oxide: 1.0 μg/L) (National Center for Health Statistics, 2011a, 2011b, 2009a, 2007). For total arsenic and speciated arsenics, urine samples below LOD were assigned a value equal to LOD/√2. Given that the majority of participants had arsenous acid, arsenic acid and arsenocholine below their respective LODs, urinary inorganic arsenic (iAs) was estimated by subtracting arsenobetaine from total arsenic.

Fasting glucose, insulin and HOMA2-IR

Participants scheduled for the morning MEC session who were 12 years and older were asked to fast overnight. Serum glucose was measured using the enzyme hexokinase method on a Cobas Mira Chemistry System (Roche Diagnostic Systems, Inc., Montclair, NJ) at University of Missouri-Columbia in 2003/2004 (Department of Child Health University of Missouri-Columbia, 2003) and on a Roche/Hitachi 911 Analyzer (Roche Diagnostics, Indianapolis, IN) at the University of Minnesota Medical Center in 2005/2006 (University of Minnesota Medical Center, 2008). Between 2007 and 2010, serum glucose was measured using Roche/Hitachi Modular P Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN) at the University of Minnesota Medical Center (University of Minnesota Medical Center, 2009). Following analytic guidelines from NHANES, serum glucose concentrations from 2003/04 and 2005/06 were calibrated to measurements conducted from 2007 onwards (National Center for Health Statistics, 2010, 2008). Serum insulin was measured using two-site immunoenzymometric assay on a Tosoh AIA-600II (TOSOH Bioscience Inc., South San Francisco, CA) at University of Missouri-Columbia in 2003/04; using the Mercodia insulin ELISA system (Mercodia AB, Uppsala, Sweden) between 2005 and late 2009; and using chemiluminescent immunoassay on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation, Indianapolis, IN) between late 2009 and 2010 at University of Minnesota Medical Center (National Center for Health Statistics, 2012a, 2008). Insulin concentrations were therefore calibrated to measurements conducted in 2009.

Participant's fasting glucose and insulin values were used to calculate their HOMA2-IR (insulin resistance) value using the HOMA Calculator software (Diabetes Trial Unit, the Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, n.d.) Like the original HOMA, HOMA2 estimates insulin resistance based on a hypothesized hepatic-beta cell feedback loop that determines basal glucose and insulin levels (Diabetes Trial Unit, the Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, n.d.). However, HOMA2 accounts for variations in hepatic and peripheral glucose resistance and is calibrated for use with currently available insulin assays (Diabetes Trial Unit, the Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, n.d.). HOMA-IR has been validated as a measure of insulin resistance in adolescents (Conwell et al., 2004; Gungor et al., 2004). For the HOMA Calculator to output the most accurate and reliable HOMA2-IR values, the ideal input ranges of glucose and insulin are 3.5 to 25 mmol/L (63 to 450 mg/dL) and 20 to 400 pmol/L (3.3 to 66.7 μU/mL), respectively, because these values are considered clinically realistic in a fasting adult (Diabetes Trial Unit, the Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, n.d.). The HOMA Calculator failed to return the HOMA2-IR value of one participant, because their fasting glucose (8.55 mmol/L, equivalent to 153.9 mg/dL) and insulin (1004.53 pmol/, equivalent to 167.4 μU/mL) profile severely violated the model's homeostatic assumption. Among participants in the final data set, all had HOMA2-IR values, but 14 (1.7%) of them had insulin values out of the ideal range. In spite of this situation, these 14 individuals were included in all analyses and their impact was addressed in sensitivity analyses.

Other Covariates

Creatinine concentration of each urine sample was obtained to account for urine dilution. Creatinine was measured on a Beckman CX3 using the Jaffe reaction prior to 2007. From 2007 onwards, creatinine was measured on a Roche ModP Chemistry Analyzer with an enzymatic method. As recommended by NHANES, urinary creatinine concentrations were calibrated to measurements conducted from 2007 onwards (National Center for Health Statistics, 2009b). Demographic variables, including age, sex, race/ethnicity and annual family income, were collected at in-home interviews. Annual family income was then used to calculate "Poverty-income-ratio (PIR)", which is defined as the ratio of family income to poverty threshold as determined annually by the United States Department of Health and Human Services (National Center for Health Statistics, 2009c). Throughout the analysis, PIR was used as a socioeconomic status indicator. Participants' weight, height and waist circumference were measured at the MEC exam. BMI was calculated as weight(kg)/ height(m)^2. Exposure to cigarette smoke was captured by serum cotinine, which was measured in serum using HPLC coupled to APCI tandem mass spectrometry (National Center for Health Statistics, 2012b). For descriptive purposes, smokers were defined as participants whose serum cotinine >= 10 ng/mL (Centers for Disease Control and Prevention, 2013; Navas-Acien et al., 2008; Pirkle et al., 1996)

Statistical Analysis

Descriptive statistical analyses were conducted in SAS 9.3 (SAS Institute Inc., Cary, NC) and regression model fitting was conducted in R (R version 3.1.0. Released on April 10, 2014). The complex sampling design was ignored because the study population belonged to two subsamples of NHANES (environmental subsample A and fasting subsample). Using the sampling weight of either subsample is not recommended by NHANES and could result in unreliable estimates (Trasande et al., 2013). The significance level of all statistical tests was set at p=0.05.

The distributions of insulin, HOMA2-IR, all arsenic measures, urinary creatinine and serum cotinine were right-skewed. HOMA2-IR was log-transformed for subsequent statistical analyses. For descriptive purposes, the medians of continuous variables and the sample proportions of categorical variables were obtained. Concentrations of total arsenic, inorganic arsenic, DMA and HOMA2-IR by participant characteristics were compared using Wilcoxon Rank Sum Test or Kruskal-Wallis Test.

To estimate the association between HOMA2-IR (log-transformed) and each arsenic marker, we employed generalized additive mixed models with random effects for sampling clusters, progressively adjusting for covariates. Generalized additive mixed models were used to optimize model fitting using splines and to account for intra-cluster correlation. We fit regression models to continuous arsenic markers and quartiles of arsenic markers. Model 1 was adjusted for urinary creatinine to account for urine dilution (creatinine entered as a covariate), as well as potential confounders based on prior knowledge, including age, sex, race/ethnicity, PIR and serum cotinine (American Cancer Society, 2014; Davis et al., 2012; Lee et al., 2006); Model 2 was further adjusted for BMI and waist circumference, two strong predictors of insulin resistance; Model 3 was additionally adjusted for arsenobetaine to

delineate potential negative confounding by non-toxic arsenic exposure (Longnecker, 2009; Navas-Acien et al., 2009, 2008; Steinmaus et al., 2009). Among all covariates, BMI was fitted with penalized splines due to its non-linear relationship with log-transformed HOMA2-IR. Continuous arsenic measures and other continuous covariates were fitted in their original forms because their penalized splines indicated linear associations with the response variable. Despite some collinearity, we decided to retain both BMI and waist circumference in the fully-adjusted models (model 3) because models including both variables had the best adjusted R-squares compared with models that adjusted for only either of them (In these models, arsenic marker was continuous and all other covariates were included. The beta coefficients of arsenic markers were similar regardless of whether BMI, waist circumference, or both were adjusted). To report regression results, we computed percent changes in HOMA2-IR associated with one interquartile range (IQR) increase in each arsenic species, and comparing each of the upper three quartiles with the first quartile. Finally, we examined potential effect modification by age and sex in the fully adjusted model (Model 3) by adding an interaction term between the continuous arsenic marker and age (12-15 vs. 16-19 years) or sex for each type of arsenic.

As a sensitivity analysis, we re-ran the fully-adjusted models after excluding individuals outside the ideal glucose and insulin ranges (3.5 to 25 mmol/L for glucose and 20 to 400 pmol/L for insulin). We also re-ran the fully-adjusted models after adding individuals fasting for less than eight hours back to our analytic sample. Additionally, we examined the arsenic-insulin resistance associations using the old version of HOMA-IR, calculated with the following formula: “(fasting glucose fasting insulin)/22.5” (fasting glucose in mmol/L and fasting insulin in mU/L) (Wallace et al., 2004). Lastly, we fit fasting glucose as the outcome in Model 3 to examine potential arsenic-fasting glucose associations.

Results

The median age (25th percentile (Q1), 75th percentile (Q3)) of the study population was 15 (13, 17) years (Table 1). The medians (Q1, Q3) of fasting glucose and fasting insulin were 94.0 (89.2, 98.2) mg/dL and 10.4 (7.16, 15.64) μU/mL, respectively. Almost all participants (99.4%) had urinary total arsenic above LOD. Proportions of participants with DMA and arsenobetaine above LODs were 86.4% and 51.4%. Median total arsenic, inorganic arsenic and DMA concentrations in the urine were 7.01 (4.13, 12.80) μg/L, 5.90 (3.40, 9.90) μg/L and 3.77 (2.17, 5.58) μg/L respectively. The median HOMA2-IR was 1.20 (0.8, 1.80) (Table 2).

Total arsenic, inorganic arsenic and DMA were distributed in similar patterns across the covariate strata (Table 2). Specifically, compared with their respective reference group, 16-to-19-year-olds, males, other Hispanic and other races, subjects in poverty (PIR<1) and those with serum cotinine ≥10 ng/mL had the highest median total arsenic, inorganic arsenic and DMA, although in some instances, the difference was not statistically significant. Subjects whose BMI and waist circumference were at or above the 95th percentile of each had the lowest median total arsenic, inorganic arsenic and DMA, but the differences were not statistically significant. Mexican American, girls and people of lower socioeconomic

status tended to have higher HOMA2-IR values (Table 2). BMI and waist circumference were positively associated with HOMA2-IR.

When adjusting for urine dilution and demographic variables only, total arsenic, inorganic arsenic and DMA were inversely associated with HOMA2-IR (Table 3, Model 1). These associations became positive after adjusting for BMI and waist circumference, but remained small in magnitude (Table 3, Model 2). Further adjusting for arsenobetaine heightened the association between HOMA2-IR and total arsenic, but the association did not reach statistical significance. For inorganic arsenic and DMA, arsenobetaine adjustment had little impact (Table 3, Model 3). In the fully-adjusted models, interquartile range increases in total arsenic, inorganic arsenic and DMA were associated with 1.5% (95% CI: -2.0, 5.2), 1.1% (95% CI: -1.5, 3.8) and 0.25% (95% CI: -2.3, 2.9) increase in HOMA2-IR, respectively. Quartiles of arsenic species were not associated with HOMA2-IR, although in the fully-adjusted model, the fourth quartile of inorganic arsenic was associated with a suggestive, 9.3% (95% CI: -2.4, 22) increase in HOMA2-IR. Overall, we found no associations between HOMA2-IR and total arsenic, inorganic arsenic or DMA.

No significant effect modification by age or sex was found (results not shown). In sensitivity analyses, restricting regression analyses to subjects with glucose and insulin values within the ideal ranges did not alter our conclusions. Adding subjects who fasted for less than eight hours back to the analytic sample resulted in slightly weaker associations for all three arsenic species. Results similar to those reported in Table 3 were found when the old version of HOMA-IR was used as the response variable. Fasting glucose was not associated with arsenic measures.

Discussion

This is the first study to investigate the association between arsenic and insulin resistance in US adolescents. After adjusting for potential confounders, including measures of obesity (BMI and waist circumference) and non-toxic arsenic species (arsenobetaine), we found no association between HOMA2-IR and total arsenic, inorganic arsenic or DMA. Despite arsenic's association with diabetes in adults and potential role in insulin resistance, our findings do not support the hypothesis that arsenic exposure at levels common in the U.S. contributes to insulin resistance in adolescents.

One obvious interpretation for the null finding is this population's low dose and short duration of exposure. The median age of this population was 15 years and their median total urinary arsenic was 7.01μg/L. Compared to the study by Lin et al., where a positive association was found between arsenic and insulin resistance in children (Lin et al., 2014), this NHANES population was a bit older (15 years in this study vs. 8.8 years in Lin et al.'s), but exposed to much lower levels of arsenic (mean total urinary arsenic of 12.7 μg/L in this study vs. 24.5 μg/L in Lin et al.'s). The inconsistency between our findings and Lin et al.'s may imply a potentially non-linear dose-response relationship between arsenic and insulin resistance. In other words, appreciable changes in insulin resistance in response to arsenic may occur only after intense exposure for a longer period of time.

Alternatively, our results may suggest that instead of insulin resistance, impaired insulin secretion is a more important mechanism underlying the association between arsenic and diabetes. The lack of association between arsenic and insulin resistance we observed is consistent with previous findings in adults. Specifically, one study conducted among American Indians found no association between arsenic exposure and insulin resistance in non-diabetic subjects, but a positive association between arsenic and diabetes prevalence (Gribble et al., 2012). Another study conducted among primarily adult residents of two arsenicosis-endemic areas of Mexico found a positive association between arsenic exposure and diabetes prevalence, but an inverse association between arsenic exposure and insulin resistance (measured by HOMA-IR) (Del Razo et al., 2011). Yet another study among Korean adults demonstrated increased odds of diabetes with increasing concentrations of urinary arsenic, but no association between arsenic and insulin sensitivity (measured by HOMA2-%S, which is the reciprocal of HOMA2-IR) in non-diabetics or drug-naïve diabetics (Rhee et al., 2013). We were unable to link arsenic exposure to clinical endpoints in our study, but observed a similar null association between arsenic and insulin resistance in a much younger population. Given that both insulin resistance and impaired insulin secretion are potential mechanisms for arsenic-related diabetes, and that the majority of population studies thus far have not been able to demonstrate positive associations between arsenic and insulin resistance, whether arsenic leads to diabetes primarily through impaired insulin secretion warrants further investigation.

A third interpretation for the null finding pertains to misclassification of exposure. Arsenic in this study was measured in a spot urine sample, but among adults with chronic arsenic exposure, the intraclass (within-subject) correlation coefficient of repeated urinary arsenic measurements has been found to be around 0.50 (Kile et al., 2009), a moderate within-subject consistency. Moreover, in terms of classifying levels of long-term exposure to arsenic (high vs. low), Kile et al. also showed that the sensitivity and specificity of one spot urine sample were both around 0.70 (Kile et al., 2009). Measuring arsenic in one spot urine sample may therefore generate some exposure misclassification, potentially biasing results towards the null (Rothman et al., 2008). Furthermore, insulin resistance or deficiency may be related to insults during fetal development (Osmond and Barker, 2000), and *in utero* exposure to arsenic has been linked to adverse cognitive outcomes in children (Hamadani et al., 2011). If instead of cumulative exposure, arsenic exposure *in utero* is more important in determining insulin resistance or impaired insulin secretion, measuring arsenic in one spot urine sample during adolescence may also misclassify the most relevant exposure. Due to the study's cross-sectional nature and limited data availability, it is difficult to settle on any one of the interpretations above. Longitudinal studies at diverse exposure levels should help clarify the role of arsenic in insulin resistance or insulin secretion impairment.

We adjusted for creatinine in our statistical models, but such an approach has been a concern when investigating the association between urinary arsenic and diabetes in cross-sectional studies, because diabetes may lead to kidney dysfunction, which could affect creatinine excretion (Maull et al., 2012). While type-2 diabetes is a major risk factor of chronic kidney disease (National Kidney Foundation, 2015), whether insulin resistance causes kidney dysfunction is controversial (Mak, 2008). Without adjusting for creatinine, the associations

between arsenic markers and HOMA2-IR all became negative but remained small and statistically non-significant (results not shown). Rather than reverse causation, these changes may simply reflect that urine creatinine is an important confounder of the arsenic-insulin resistance association. Urine creatinine is an indirect measure of skeletal muscle mass; and relative muscle mass has been found inversely associated with insulin resistance (Poortmans et al., 2005; Srikanthan and Karlamangla, 2011). Because accounting for urine dilution is necessary for valid inference using urine biomonitoring data, in the absence of better alternatives, we adjusted for urine creatinine. Interpretations of our findings should consider the impact of creatinine adjustment.

Other limitations of this study include the temporal ambiguity inherent in the cross-sectional design. In addition, pubertal status is a strong predictor of insulin resistance in adolescents, but due to limited data availability in NHANES, we were unable to stratify on pubertal status to assess the association between arsenic and HOMA2-IR. Moreover, arsenic species below their respective limit of detection (LOD) were imputed with LOD/√2. This approach may lead to incorrect inferences under certain scenarios, especially when the proportion of samples below LOD is large (which is the case for arsenobetaine in our analysis.) (Schisterman et al. 2006). Unfortunately, for biomarkers below LODs, NHANES provides only the imputed values of LOD/√2. Our analysis was thus limited by NHANES' imputation approach. Finally, since we ignored NHANE's complex sampling design, our results are not representative of the general U.S. adolescent population as NHANES originally intended. However, findings in this sample of adolescents still serve to expand our knowledge in arsenic's potential role in insulin resistance and diabetes.

Conclusions

In conclusion, in a population of U.S. adolescents with low-to-moderate arsenic exposure, we found no association between arsenic and insulin resistance. Even though arsenic has been associated with diabetes in U.S. adults, and insulin resistance has been proposed as a link between arsenic and diabetes, our findings indicate that the metal has limited impact on adolescents' insulin resistance, at least at exposure levels common in the US. Whether these results suggest that longer duration of more intense arsenic exposure is required for appreciable changes in insulin resistance, or that insulin resistance is not a major diabetogenic mechanism of arsenic needs further studies, especially longitudinal ones at diverse exposure levels and those that evaluate insulin-secretion-related endpoints.

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Table 1

Participant Characteristics

Participant Characteristics	Median (Q1, Q3) or N (%)[a]
Age (years)	15 (13, 17)
Sex	
Male	463 (55.5%)
Race/ethnicity	
Non-Hispanic white	254 (30.4%)
Mexican American	237 (28.4%)
Non-Hispanic black	262 (31.4%)
Other Hispanic and other race	82 (9.8%)
Poverty-income ratio (PIR)	1.5 (0.80, 3.11)
Participants with PIR<1	277 (33.2%)
Body mass index (BMI) (kg/m^2)	22.4 (19.9, 26.7)
Waist circumference (cm)	77.6 (71.4, 88.5)
Serum cotinine (ng/mL)	0.089 (0.021, 1.02)
Participants with serum cotinine>=10 ng/mL	103 (12.3%)
Fasting glucose (mg/dL)	94.0 (89.2, 98.2)
Fasting insulin (μU/mL)	10.4 (7.16, 15.64)

Reported as “N of participants (%)” for sex, race/ethnicity, “participants with PIR<1” and “participants with serum cotinine>=10ng/mL”.

[a]Reported as “median (Q1, Q3)” for age, PIR, BMI, waist circumference, serum cotinine, fasting glucose and fasting insulin.

Table 2

Medians of Total Arsenic, Inorganic Arsenic, DMA and HOMA2-IR by Covariates

	Median (Q1, Q3)							
	Total Arsenic (μg/L)	**p-value[a]**	**Inorganic Arsenic (μg/L)**	**p-value**	**DMA (μg/L)**	**p-value**	**HOMA2-IR**	**p-value**
All (n=835)	7.01 (4.13, 12.80)		5.90 (3.40, 9.90)		3.77 (2.17, 5.58)		1.20 (0.8, 1.80)	
Age (years)								
12-15 (n=432)	6.58 (3.81, 11.20)		5.60 (3.20, 9.09)		3.60 (2.00, 5.10)		1.25 (0.90, 1.80)	
16-19 (n=403)	7.79 (4.59, 14.90)	0.0028	6.60 (3.69, 10.87)	0.021	3.97 (2.30, 6.00)	0.036	1.10 (0.80, 1.70)	0.0095
Sex								
Male (n=463)	7.17 (4.57, 14.16)		6.20 (3.90, 10.10)		3.94 (2.30, 5.77)		1.10 (0.80, 1.70)	
Female (n=372)	6.61 (3.62, 12.49)	0.020	5.68 (2.83, 9.62)	0.027	3.60 (2.00, 5.30)	0.036	1.30 (0.90, 1.90)	0.0003
Race/ethnicity								
Non-Hispanic white (n=254)	6.36 (5.53, 11.00)		5.48 (3.00, 8.30)		3.20 (2.00, 4.85)		1.10 (0.80, 1.60)	
Mexican American (n=237)	6.90 (4.01, 11.61)		5.70 (3.25, 9.36)		3.70 (2.20, 5.47)		1.30 (0.90, 2.00)	
Non-Hispanic black (n=262)	7.90 (4.98, 15.76)		6.56 (4.01, 10.80)		4.00 (2.65, 6.00)		1.15 (0.80, 1.80)	
Others (n=82)	7.98 (4.13,17.21)	0.0004	6.91 (3.85, 14.10)	0.002	4.42 (2.48, 7.49)	0.0006	1.15(0.80, 1.60)	0.0004
PIR								
<1 (n=277)	7.80 (4.43, 14.50)		6.50 (3.62, 10.90)		3.92 (2.11, 6.00)		1.30 (0.90, 2.00)	
1-3 (n=399)	6.59 (3.82, 12.10)		5.70 (3.19, 9.50)		3.65 (2.06, 5.21)		1.10 (0.80, 1.70)	
≥3 (n=219)	6.91 (4.40, 12.47)	0.13	5.86 (3.41, 9.65)	0.20	3.67 (2.41, 5.30)	0.44	1.10 (0.80, 1.50)	0.0001
Serum Cotinine (ng/mL)								
<0.015 (n=137)	6.30 (4.13, 12.47)		5.65 (3.19, 10.46)		3.62 (2.23, 6.93)		1.20 (0.80, 1.70)	
≥0.015 and <10(n=595)	6.91 (3.97, 12.30)		5.85 (3.32, 9.50)		3.67 (2.10, 5.32)		1.20 (0.80, 1.80)	
≥10 (n=103)	8.30 (5.20,16.40)	0.027	7.30 (4.16, 11.70)	0.066	4.00 (2.50, 6.00)	0.22	1.00 (0.70, 1.50)	0.0098
BMI (kg/m^2)								
<85th percentile (n=708)	6.97 (4.11, 12.80)		5.90 (3.35, 9.90)		3.71 (2.11, 5.55)		1.10 (0.80, 1.50)	
<95th percentile (n=85)	7.55 (4.60, 12.20)		6.40 (4.32, 9.67)		4.00 (2.55, 5.70)		2.10 (1.60, 3.20)	
≥95th percentile (n=42)	6.33 (4.41, 15.10)	0.94	5.36 (3.50, 10.40)	0.80	3.64 (2.65, 5.10)	0.45	3.00 (2.50, 4.00)	<.0001
Waist circumference (cm)								
<85th percentile (n=707)	6.99 (4.13, 12.90)		5.90 (3.35, 9.71)		3.71 (2.11, 5.58)		1.10 (0.80, 1.50)	
<95th percentile (n=86)	7.67 (4.60, 13.60)		6.46 (4.32, 10.55)		4.10 (2.80, 6.00)		2.10 (1.50, 3.00)	
≥95th percentile (n=42)	6.03 (4.01, 10.20)	0.50	4.65 (2.79, 8.40)	0.32	3.04 (2.10,4.74)	0.11	3.25 (2.50, 4.10)	<.0001

[a] P-values obtained from Wilcoxon Rank Sum Test if comparing between two groups, and obtained from Kruskal-Wallis Test if comparing more than two groups.

Table 3

Percent Change in HOMA2-IR Associated with one Interquartile Range Increase or Quartiles of Arsenic Species

	Percent Change in HOMA2-IR (%) (95% CI)					
	Per Interquartile Range Increase	Quartile[a]				P-trend[c]
		1	2	3	4	
Total Arsenic						
Model 1[b]	−0.22 (−1.5, 1.1)	Ref	2.2 (−9.1, 15)	−5.4 (−17, 7.5)	−4.6 (−17, 9.0)	0.31
Model 2	0.35 (−0.65, 1.4)	Ref	1.1 (−8.0, 11)	−0.89 (−10, 9.7)	−0.047 (−10, 11)	0.91
Model 3	1.5 (−2.0, 5.2)	Ref	1.0 (−8.0, 11)	−0.99 (−11, 9.6)	−0.69 (−11, 11)	0.81
Inorganic Arsenic[d]						
Model 1	−0.59 (−3.4, 2.3)	Ref	13 (0.44, 27)	4.7 (−7.8, 19)	7.3 (−6.8, 23)	0.62
Model 2	1.1 (−1.1, 3.4)	Ref	9.0 (−0.67, 20)	6.1 (−4.1, 17)	9.6 (−1.9, 23)	0.19
Model 3	1.1 (−1.5, 3.8)	Ref	9.0 (−0.70, 20)	6.0 (−4.1, 17)	9.3 (−2.4, 22)	0.22
DMA						
Model 1	−1.3 (−4.3, 1.8)	Ref	9.9 (−2.6, 24)	6.9 (−5.8, 21)	2.6 (−11, 18)	0.95
Model 2	0.40 (−2.0, 2.9)	Ref	6.3 (−3.3, 17)	5.4 (−4.7, 16)	4.2 (−6.5, 16)	0.59
Model 3	0.25 (−2.3, 2.9)	Ref	6.3 (−3.4, 17)	5.2 (−4.8, 16)	3.9 (−7.0, 16)	0.64

[a] Total arsenic Quartile 1-4: <=4.11 μg/L, 4.13-6.99 μg/L, 7.01-12.80 μg/L, >=12.86 μg/L Inorganic arsenic Quartile 1-4: <=3.36 μg/L, 3.40-5.90 μg/L, 5.90-9.89 μg/L, >=9.90 μg/L DMA Quartile 1-4: <=2.15μg/L, 2.17-3.75 μg/L, 3.77-5.52 μg/L, >=5.58 μg/L

[b] Model1: Adjusted for urinary creatinine, age, sex, race/ethnicity, PIR and serum cotinine Model2: Model1+ BMI (penalized spline) and waist circumference Model3: Model2+ urinary arsenobetaine

[c] Based on the model where quartiles of total arsenic, inorganic arsenic or DMA are fitted as ordinal variables.

[d] Inorganic arsenic=total arsenic-arsenobetaine