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Blood Pressure Changes in Relation to Arsenic Exposure in a U.S. Pregnancy Cohort

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Abstract

Background: Inorganic arsenic exposure has been related to the risk of increased blood pressure based largely on cross-sectional studies, conducted in highly exposed populations. Pregnancy is a period of particular vulnerability to environmental insults. However, little is known about the cardiovascular impacts of arsenic exposure during pregnancy.

Objectives: To evaluate the association between prenatal arsenic exposure and maternal blood pressure over the course of pregnancy in a US population.

Methods: The New Hampshire Birth Cohort Study is an ongoing prospective cohort study, in which over 10% of participant household wells exceed the arsenic maximum contaminant level of 10 µg/L established by the US EPA. Total urinary arsenic measured at 24-28 weeks gestation was measured in 514 pregnant women, ages 18-45, who used a private well in their household and used as a biomarker of exposure during pregnancy. Outcomes were repeated blood pressure measurements (systolic, diastolic and pulse pressure) recorded during pregnancy.

Results: Using linear mixed effects models, we estimated that, on average, each 5 µg/L increase in urinary As was associated with a 0.15 mmHg (95% CI: 0.02, 0.29, p = 0.022) increase in systolic blood pressure per month and a 0.14 mmHg (95% CI: 0.02, 0.25; p=0.021) increase in pulse pressure per month over the course of pregnancy.

Conclusions: In our US cohort of pregnant women, arsenic exposure was associated with greater increases in blood pressure over the course of pregnancy. These findings may have important implications as even modest increases in blood pressure impact cardiovascular disease risk.
Introduction

Millions of individuals are chronically exposed to inorganic arsenic via contaminated water sources and through diet (National Research Council 2014; Navas-Acien and Nachman 2013). In the US, an estimated 17 million people have been exposed to drinking water sources containing arsenic levels exceeding the maximum contaminant limit of 10 µg/L (U.S. Environmental Protection Agency 2000). Common dietary staples, such as rice and poultry, have been found to contain elevated levels of arsenic that also contribute to an individual’s overall exposure (Cottingham et al. 2013; Davis et al. 2012; Gilbert-Diamond et al. 2011; Nachman et al. 2013; Navas-Acien and Nachman 2013). Arsenic exposure has been associated with adverse health effects, including cancer, diabetes, and cardiovascular disease (National Research Council 2014).

Cardiovascular disease is the leading cause of morbidity and mortality worldwide (World Health Organization 2008) and associations between arsenic and the risk of cardiovascular events have been well documented in highly exposed populations (Chen et al. 2011; Moon et al. 2012; Moon et al. 2013; States et al. 2009). Recent prospective work in the US observed a relation between low level arsenic exposure and risk of cardiovascular disease (Moon et al. 2012; Moon et al. 2013). Indeed, a growing body of evidence suggests that arsenic may increase risks of some risk factors for cardiovascular diseases, including high blood pressure, atherosclerosis and endothelial dysfunction (Chen et al. 2007a; Chen et al. 2007b; Chen et al. 2013; Hsieh et al. 2011; Wang et al. 2007; Wu et al. 2012). However, available evidence on cardiovascular disease risk factors is based on cross-sectional studies and prospective studies that characterize the magnitudes of longitudinal changes in risk factors related to arsenic exposure are lacking. Moreover, certain populations, such as pregnant women, may be especially susceptible to these adverse effects, yet little is known about the cardiovascular effects of arsenic exposure during this time period.
Pregnancy profoundly alters both maternal anatomy and physiology to support fetal development (Cunningham et al. 2010). Pregnancy-induced hemodynamic adaptations and hormonal changes lead to normal fluctuations in gestational blood pressure (Cunningham et al. 2010). However, these changes can act as cardiovascular and metabolic stressors (Yoder et al. 2009), creating a “susceptible window” of risk for development of hypertension from putative triggers, including environmental exposures such as lead and air pollutants (Jedrychowski et al. 2012; Lee et al. 2012; van den Hooven et al. 2011; Yazbeck et al. 2009). Further, high blood pressure during pregnancy can signal a greater risk of later life maternal cardiovascular disease (Henriques et al. 2014; Irgens et al. 2001; Magnussen et al. 2009; Nisell et al. 1995; Skjaerven et al. 2012; Wilson et al. 2003) and also enhances risk of adverse birth outcomes such as premature labor, placental abruption, and restricted placental blood flow to the fetus, which is related to low birth weight (Allen et al. 2004; Roberts et al. 2005).

In New Hampshire, about 40% of households rely on unregulated private water systems, of which 10-15% contain arsenic levels exceeding the maximum contaminant level (Karagas et al. 2002). As part of the New Hampshire Birth Cohort Study, we sought to investigate whether higher maternal arsenic exposure during pregnancy is related to increases in maternal blood pressure, an early cardiovascular disease risk factor and a complicating factor in pregnancy.

**Methods**

*The New Hampshire Birth Cohort:* In January 2009, we began recruiting 18-45 year old pregnant women receiving prenatal care at study clinics, as previously described (Gilbert-Diamond et al. 2011). Women were enrolled at 24-28 weeks gestation if they reported using water from a private well at their residence since their last menstrual period and were not planning to move prior to delivery. Only singleton births are included in the study. All protocols
were approved by the Dartmouth College Institutional Review Board. All participants provided written, informed consent upon enrollment.

Participants completed a detailed medical history and lifestyle questionnaire upon enrollment and a follow-up questionnaire at two weeks postpartum to obtain updated information about changes in key exposures and prenatal complications. After delivery, participants’ medical records were reviewed to abstract pre- and post-delivery health information, including all clinically measured maternal blood pressure levels, diagnoses of gestational diabetes, hypertension, pre-eclampsia and eclampsia, Other clinical information was recorded to verify self-reported medical and reproductive history. Maternal systolic (SBP) and diastolic (DBP) blood pressure was measured in the study clinics using either automated or mercury sphygmomanometers throughout pregnancy and generally recorded at each prenatal visit.

*Arsenic Exposure Assessment:* Women provided a spot urine sample upon enrollment, which was collected and stored, as previously described (Farzan et al. 2013; Gilbert-Diamond et al. 2011). Urines were analyzed for levels of arsenite (iAs$_{\text{III}}$), arsenate (iAs$_{\text{V}}$), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine by high-performance liquid chromatography (HPLC) inductively coupled plasma mass spectrometry (ICP-MS) at the University of Arizona Hazard Identification Core (Larsen et al. 1993; Le et al. 2000; Wei et al. 2001). Samples that registered below the detection limit (ranging from 0.10-0.15 μg/L for individual species; 0.6%, 16.5%, and 37.0% of the study population were below the detection limit for DMA, MMA and iAs, respectively) were assigned a value equal to the detection limit divided by the square root of two. Urinary creatinine levels (mg/dL) were determined using Cayman's creatinine assay kit, according to manufacturer’s instructions. Our primary exposure measure was total urinary arsenic at 24-28 weeks gestation, calculated by summing inorganic
(iAs = iAs^{III}+iAs^{V}) and organic (DMA, MMA) metabolites (Farzan et al. 2013; Gilbert-Diamond et al. 2011). Arsenobetaine, an unmetabolized form of arsenic found in seafood was excluded, as it is considered non-toxic (Tseng 2009). As secondary exposure measures, we examined the absolute values of urinary metabolites (MMA, DMA, iAs). We also constructed primary (PMI) and secondary methylation indices (SMI) from ratios of MMA to iAs and DMA to MMA in urine, respectively, as these are considered indicators of methylation capacity that may impact individual variability in health effects of arsenic exposure (Chen et al. 2013). Participants also were given instructions and prepaid mailing materials upon enrollment to collect samples of their home tap water and return the samples to the study office, which were analyzed by ICP-MS at the Dartmouth Trace Element Analysis Core, as previously described (Gilbert-Diamond et al. 2011). Maternal toenail samples were collected at two weeks postpartum, washed 5 times by sonication in a solution of Triton X-100 and acetone, followed by deionized water and then dried before low-pressure microwave digestion. Samples were analyzed for trace elements previously related to BP (i.e., Se, Cd, Fe, Hg, and Pb) (Houston 2007; Kennedy et al. 2012; Wells et al. 2012) using ICP-MS as previously described for arsenic (Davis et al. 2014).

**Statistical Analysis:** We confined our analysis to women without a history of hypertension prior to pregnancy with at least two pregnancy blood pressure measurements. Our outcomes of interest were temporal changes in SBP, DBP and pulse pressure (PP: SBP minus DBP) during pregnancy, which were analyzed as continuous variables with repeated measurements. For each measurement, we calculated the trimester and gestational week, based on the participant’s last menstrual period. We restricted our analysis to measurements taken after 13 weeks gestation due to the few number of measurements recorded before this time. Measurements outside of a reasonable range (i.e., SBP: <40 or >250mmHg, DBP: <35 or >180mmHg) (Lee et al. 2012)
which were likely incorrectly recorded at time of measurement or incorrectly extracted from the medical record, as all values that were excluded were well outside of the physiologically plausible range, were coded as missing (<1% of measurements, n=9). All other values recorded for these women were within a physiologically reasonable range. There were few cases of diagnosed pregnancy-induced hypertension (n=15) or preeclampsia (n=9) in our study population, thus it was not possible to analyze these outcomes separately.

We fitted mixed effect models (Demidenko 2004) of the repeated blood pressure measurements to examine whether maternal urinary total arsenic or arsenic metabolite concentrations influenced SBP, DBP, and PP over the course of pregnancy, as follows:

\[
BP_{ij} = [\beta_0 + \beta_1(TIME)_{ij} + \beta_2 As_{0j} + \beta_{12} As_{0j}(TIME)_{ij} + \alpha^T Z_{0j}] + [\mu_{0j} + \mu_{ij}(TIME)_{ij}] + r_{ij} \quad [1]
\]

Where \(BP_{ij}\) represents blood pressure at time \(i\) for subject \(j\), \(As_{0j}\) is urinary arsenic (total, DMA, MMA or iAs) at baseline (time 0 represents baseline, i.e. the gestational month of each woman’s first blood pressure measurement after 13 weeks gestation) for subject \(j\); \(TIME\) is gestational month of BP measurement; \(\beta_1\) is the coefficient for the association between \(TIME\) and \(BP\) when arsenic is held constant; \(\beta_2\) is the difference in blood pressure for every unit increase in arsenic at baseline; \(\beta_{12}\) is the difference in monthly blood pressure change over pregnancy per unit increase in arsenic (i.e. the estimated effect of arsenic levels on monthly blood pressure change); \(\alpha^T\) is a row vector of regression coefficients for covariates at baseline (T denotes vector transpose); \(Z_{0j}\) is a vector of covariates at baseline. The random intercept \(\mu_{0j}\) and slope \(\mu_{ij}\) estimated the within-subject correlation among repeated measurements and between-subject heterogeneity, and \(r_{ij}\) is the error that cannot be accounted for by other covariates and random effects. The terms in the first and second brackets are the fixed and random parts of the model, respectively. We assessed nonlinear trends in the data using the same modeling strategy described above and including
model terms to examine the interaction between TIME and categories of arsenic exposure variables (e.g., dummy variables for arsenic tertiles), as well as linearity of the time effect by including an additional interaction term between As$_{0j}$ and TIME$^2$. Neither test provided evidence of a nonlinear association ($p > 0.05$). For ease of interpretation, 5µg/L (~1 SD) was used as the unit to report effect estimates for total urinary arsenic and metabolite levels.

Our models were adjusted for available covariates that could potentially influence blood pressure based on a priori considerations, including age at enrollment, pre-pregnancy body mass index (BMI), smoking during pregnancy, marital status, educational attainment, gestational diabetes, parity, and number of blood pressure measurements. As described above, we included the month of gestation during which each blood pressure measurement was obtained in our models. We considered pregnancy blood pressure measurements after 13 weeks gestation (our baseline) in our models, as few subjects received blood pressure measurements prior to that time point.

Urinary arsenic concentrations were used a measure of gestational arsenic exposure, since urine samples earlier in pregnancy were not available and prior studies suggest that total arsenic concentrations remain relatively stable (Ahmed et al. 2011; Gamble et al. 2006). As there is some debate as to whether creatinine adjustment is appropriate for urinary arsenic measures, we also tested models with and without urinary creatinine adjustment. We also tested inclusion of arsenobetaine levels as a covariate in our models. We found that neither creatinine nor arsenobetaine adjustment altered our estimates, such that results were unchanged with or without creatinine adjustment (i.e., SBP $\beta_{12} 0.15$, 95% CI: 0.02, 0.29 with creatinine adjustment), as well as with or without arsenobetaine adjustment (i.e., SBP $\beta_{12} 0.15$, 95% CI: 0.02, 0.29 with arsenobetaine adjustment) (data not shown). For individuals with missing covariate data (Table 1), we used multiple imputation to estimate missing covariate values (Little and Rubin 2002).
We examined the missing data patterns and in our models assumed that the data were missing at random with a monotone structure. We used the regression method within the SAS PROC MI procedure to generate 5 imputed datasets, then used the PROC MIANALYZE procedure to generate inferences for both the mixed and linear regression models. We also performed sensitivity analyses by excluding those who smoked during pregnancy or those who developed gestational diabetes to evaluate the impact on our results, as blood pressure may be altered in these groups (Bakker et al. 2010; Bryson et al. 2003; Carpenter 2007; Matkin et al. 1999). We also assessed other exposures from toenail levels as potential confounders. Toenail elements that have been associated with BP in the literature, such as Se, Cd, Fe, Hg, and Pb (Houston 2007; Kennedy et al. 2012; Wells et al. 2012), all had little to very weak correlations with toenail arsenic (r < 0.20) (data not shown) and thus were not adjusted for in our analysis.

We conducted analyses stratified by PMI or SMI, using the median values (0.89 and 9.66 respectively) as cutpoints, to assess whether the association between urinary arsenic and blood pressure changes over time differed by these arsenic methylation indices. We also performed analyses stratified by age (below or at/above median 30.9 years), history of prior pregnancy (nulliparous or parous), pre-pregnancy BMI (below or at/above 25 kg/m²).

As blood pressure increases over the latter part of pregnancy (Cunningham et al. 2010; Miller et al. 2007; Thompson et al. 2007), we further examined whether women with higher urinary arsenic had higher blood pressure at the end of pregnancy, using linear regression models with the outcome respectively defined as the average of the last 3 blood pressure measurements (SBP, DBP, PP), adjusting for the same covariate variables. The equation generated from the multivariable linear regression model also was used to graphically represent the relationship between maternal urinary arsenic and SBP at the end of pregnancy, when all covariates are set
equal to the median values (Figure 1). In all analyses, p-values less than 0.05 were considered significant. All analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

**Results**

As of October 30, 2013, 620 participants had available urinary arsenic measurements, and of these, 590 had available medical record review data. As an *a priori* selection requirement, we required women to have at least two blood pressure measurements taken during pregnancy; however, all 527 women in this sample had a minimum of four measurements. An additional 13 women with a history of hypertension were excluded, resulting in a final sample size of 514. This subset was similar to the overall cohort (n=620) with respect to demographic and lifestyle variables (data not shown). Women in this sample had urinary arsenic concentrations ranging from 0.35 to 288.5 µg/L, which was very similar to the range observed in the overall cohort (0.08 to 288.5 µg/L). Of the subset of participants included in these analyses, 472 had provided water samples, of which 463 had been analyzed at the time of this study and had a mean arsenic level of 4.3µg/L (range: 0-147.7µg/L). Nearly 1 in every 8 households (58 of 463 available samples; 12.5%) tested in this subsample had water arsenic levels above 10µg/L. In our study group, well water As levels were significantly correlated with urinary arsenic measurements (r=0.40, p<0.001). No differences in the descriptive variables were observed across urinary arsenic tertiles by chi-squared or one-way ANOVA tests, except for water arsenic levels, in which the third tertile was higher than the first tertile (Table 1).

The 514 women in this subsample of the cohort contributed a total of 6,675 SBP and 6,671 DBP measurements (5,773 SBP and 5,769 DBP after gestational week 13). On average, 13 (range: 4-24) blood pressure measurements were recorded per participant during pregnancy, with over half occurring in the last trimester. Both SBP and DBP increased during pregnancy (Figure 1 and see
supplemental material, Table S1), with the highest averages for both SBP and DBP occurring in the third trimester (SBP trimester mean (SD): first: 112.9 (9.8), second: 112.5 (8.2), third: 115.3 (8.4); DBP trimester mean (SD): first: 68.4 (7.7), second: 67.0 (6.0), third: 69.4 (6.2)). PP also appeared to increase over the course of pregnancy, with somewhat more variability (Figure 1 and see supplemental material, Table S1).

We did not observe any association between urinary arsenic and differences in DBP change over pregnancy. Arsenic exposure was related to greater monthly increases in SBP and PP change over the course of pregnancy (Table 2). Based on our model, each 5µg/L increase in urinary arsenic was associated with a 0.15 mmHg greater monthly increase in SBP (95% confidence interval (95% CI): 0.02, 0.29; p=0.022) and a 0.14 mmHg greater monthly increase in PP (95% CI: 0.02, 0.25; p=0.021) over the course of pregnancy (Table 2). In sensitivity analyses, excluding smokers or individuals with gestational diabetes, did not appreciably alter any of these findings (data not shown). All metabolites MMA, DMA and iAs were positively associated with greater increases in PP over the course of pregnancy (MMA $\beta_{12}$ 1.54, 95% CI: 0.16, 2.92; DMA $\beta_{12}$ 0.15, 95% CI: 0.02, 0.29; iAs $\beta_{12}$ 1.18, 95% CI: -0.01, 2.38) (Table 2). Higher levels of DMA were also associated with greater increases in SBP ($\beta_{12}$ 0.18, CI: 0.02, 0.33) over the course of pregnancy.

Women with higher PMI had greater average increases in both SBP and PP over the course of pregnancy compared to those with lower PMI, though the differences from women with lower PMI were not significant (SBP $\beta_{12}$ 0.23, 95% CI: 0.07, 0.39 versus $\beta_{12}$ 0.06, 95% CI: -0.17, 0.29; p-interaction=0.21; PP $\beta_{12}$ 0.18, 95% CI: 0.03, 0.34 versus $\beta_{12}$ 0.10, 95% CI: -0.10, 0.29; p-interaction=0.47) (Table 3). Similarly, those with higher SMI appeared to have greater increases in SBP (high SMI $\beta_{12}$ 0.25, 95% CI: 0.07, 0.42 versus low SMI $\beta_{12}$ 0.05, 95% CI: -0.15, 0.26; p-
interaction=0.17) over the course of pregnancy, although the test for interaction was not significant. No effect modification by SMI was observed for PP (high SMI $\beta_{12}$ 0.16 95% CI: 0.01, 0.31 versus low SMI $\beta_{12}$ 0.12, 95% CI: -0.06, 0.31; p-interaction=0.74). In analyses stratified by potential effect modifiers, including pre-pregnancy BMI, age, and parity, we did not observe any statistically significant associations between total urinary arsenic and longitudinal changes in blood pressure (data not shown). We also examined whether individuals with missing data impacted the outcomes and found that when individuals with missing covariate information (n= 78) were excluded that the estimates were nearly unchanged (i.e., SBP $\beta_{12}$ 0.15, 95% CI: 0.02, 0.28) (data not shown).

When we conducted simple linear regression models, based on the average of the last three blood pressure measurements, each 5µg/L increase in urinary arsenic was associated with a 0.78 mmHg (95% CI: 0.05, 1.51; p=0.035) higher SBP. Again, total urinary arsenic was unrelated to DBP ($\beta$ 0.34, 95% CI: -0.20, 0.89; p=0.22). Likewise, as in the longitudinal analysis, PP was positively associated with urinary arsenic, but with wide confidence intervals ($\beta$ 0.44, 95% CI: -0.10, 0.97; p=0.11). Graphical representation of the linear regression model depicts an increase in the average of the last three SBP measurements in relation to urinary arsenic level (see supplemental material, Figure S1).

**Discussion**

To our knowledge, our study is the first prospective study to examine the association between arsenic and blood pressure in the context of pregnancy and among the few studies on the cardiovascular effects of arsenic exposure in the US. As pregnancy is a vulnerable window of susceptibility to adverse blood pressure changes, focusing on a cohort of pregnant women we
found that higher levels of urinary arsenic during pregnancy prospectively related to greater increases in SBP and PP over the course of pregnancy.

Arsenic has been associated with a range of cardiovascular outcomes in populations with appreciable levels of chronic exposure, such as Bangladesh and Taiwan, including increased risks of fatal and non-fatal cardiovascular disease, as well as intermediary factors, such as increased carotid intima-media thickness and metabolic syndrome (Chen et al. 1995; Chen et al. 2007a; Chen et al. 2011; Chen et al. 2013; Kwok et al. 2007; Wang et al. 2007; Wu et al. 1989). Arsenic has been associated with hypertension in a number of cross-sectional studies, from which a meta-analysis derived a pooled odds ratio for hypertension of 1.27 (95% CI: 1.09, 1.47) for high versus low arsenic exposure (Abhyankar et al. 2012). While potential causal mechanisms for the association between arsenic and blood pressure increase during pregnancy have not yet been explored, many of the mechanisms hypothesized to explain associations with other cardiovascular outcomes could be involved. Arsenic exposure has been related to increased plasma markers of inflammation and endothelial damage (Burgess et al. 2013; Chen et al. 2007b; Wu et al. 2012), suggesting arsenic may act in part by promoting endothelial dysfunction, pathologic vascular remodeling and atherosclerosis. Thus, while speculative, arsenic exposure could impact the pregnancy-related hemodynamic adaptations that increase blood volume and maintain placental perfusion, which is critical to fetal nutrient and oxygen supply.

Blood pressure normally increases towards the latter part of pregnancy, with increases in SBP generally tending to be somewhat more pronounced than those in DBP (Cunningham et al. 2010; Miller et al. 2007; Thompson et al. 2007). A prospective study of longitudinal blood pressure during pregnancy reported average increases of about 3.7mmHg and 2.2mmHg between the first and third trimesters, for SBP and DBP respectively (Miller et al. 2007). Abnormal increases pose
a serious risk of complications during pregnancy such as preterm birth, low birth weight, fetal growth restriction, and perinatal mortality (Ray et al. 2001; Xiong and Fraser 2004; Zhang et al. 2007) and the deleterious effects of gestational hypertension (defined as new onset of SBP >140 mmHg and/ or DBP >90 mmHg in second trimester) are well known. However, elevations in blood pressure that do not exceed the upper threshold of the normal range (SBP <140 mmHg and DBP <90 mmHg) may also pose risks to the mother and child. For non-pregnant adults, the risk of cardiovascular disease increases linearly as blood pressure increases, even within the normotensive range (Vasan et al. 2001; Williams et al. 2008). A few studies have examined blood pressure as a continuous measure and found that higher blood pressure even within the normotensive range also may impact birth weight and intrauterine growth restriction (Churchill et al. 1997; Fukushima et al. 2012). It is possible that elevated blood pressure, albeit within the clinically normal range, alters uterine and placental perfusion, and impacts fetal growth. Our results suggest that there were greater increases in SBP and PP over pregnancy associated with higher arsenic exposure, leading to greater relative differences at the end of pregnancy. However, the clinical significance of greater increases in blood pressure remains to be explored and more studies utilizing continuous blood pressure outcome measures are needed in order to examine the relation between blood pressure elevations within the normal range and health risks.

Pregnancy itself is a cardiovascular stressor. In a rodent study normal, healthy pregnancies were found to induce long-term alterations in cardiovascular and renal function that were absent in nonparous females (Gallo et al. 2012). Pregnancy-induced hypertension has been associated with increased later life risk of chronic hypertension, endothelial dysfunction and kidney disease (Henriques et al. 2014; Nisell et al. 1995; Vikse et al. 2008; Wang et al. 2013; Wilson et al. 2003). According to a recent study, women with a history of a hypertensive pregnancy had
nearly 60 percent greater odds of peripheral artery disease compared to those with normotensive histories, even decades after pregnancy (Weissgerber et al. 2013). Additional longitudinal studies are needed to determine whether blood pressure changes during pregnancy, such as those observed in relation to arsenic exposure in our cohort, lead to long-term health consequences for mother and child.

In our study, we found that each 5µg/L urinary arsenic was associated with an average SBP increase of 0.15 mmHg per month and a 0.78mmHg (95% CI: 0.05, 1.51; p=0.035) higher SBP. While we are unaware of any previous studies of arsenic and blood pressure during pregnancy, recent studies have found that exposure to other environmental contaminants may impact blood pressure during pregnancy with similar magnitudes of effects as observed in our study. Several studies have observed associations between particulate air pollution and increased blood pressure in pregnant women (Lee et al. 2012; van den Hooven et al. 2011) including a prospective study of 431 pregnant women that found third trimester SBP increased linearly with second trimester exposure to air particulates (Jedrychowski et al. 2012). The Generation R Study found that a 10µg/m³ increase in PM₁₀ exposure was associated with greater increases in SBP over the second and third trimesters, 1.11 (95% CI: 0.43, 1.79) and 2.11 (95% CI: 1.34, 2.89) mmHg, respectively (van den Hooven et al. 2011). A recent US cohort study of air pollution on blood pressure changes over the course of pregnancy found that interquartile increases in PM₁₀ and O₃ exposure in the first trimester were associated with average increases of 1.9 mmHg in SBP (95% CI: 0.84, 2.93) and 1.8 mmHg in SBP (95% CI: 1.05, 4.63) respectively, an association which was more pronounced in nonsmoking mothers (Lee et al. 2012). Additionally, a cohort study of 1,017 pregnant women in France found an association between mid-pregnancy blood lead levels and increased risk of pregnancy-induced hypertension in the second and third trimesters.
(Yazbeck et al. 2009). While studies of the impacts of environmental toxicants on cardiovascular effects during pregnancy are growing, more studies are needed to assess the vulnerable times of exposure, as well as the effects of toxicants known to increase cardiovascular disease in non-pregnant adults, including arsenic.

Ingested inorganic arsenic is primarily metabolized via methylation, first to MMA, then DMA. Arsenic metabolism varies greatly between individuals and higher MMA proportions are indicative of inefficient methylation (Buchet et al. 1981; Vahter 1999). MMA, thought to be a more toxic metabolite, has been linked to adverse health effects, including cardiovascular effects (Chen et al. 2013; Huang et al. 2009). As previous work from more highly exposed individuals has indicated that higher PMI may be associated with greater health risks (Chen et al. 2013), one might expect to only see stronger effects in those with high PMI, which could indicate inefficient arsenic metabolism, as opposed to high SMI, which may indicate more efficient methylation and therefore arsenic excretion. However, we observed associations between urinary arsenic and blood pressure both among those with higher PMI or higher SMI, although differences may have occurred by chance. In populations with lower overall levels of exposure, one might predict that the majority of ingested arsenic, once methylated to MMA, would be more easily methylated to DMA. This prediction is consistent with our observations, as well as with those in other US populations, including recent results from the Strong Heart Study which indicated that higher DMA proportions were linked to cardiovascular disease incidence and mortality, raising the possibility for a role of higher SMI in cardiovascular risk in populations with low arsenic exposure levels (Moon et al. 2013). A low SMI may be a susceptibility factor in more highly exposed populations, such as in Bangladesh. Further, the pregnancy-related health outcomes related to high SMI (i.e. high DMA levels) are less well understood. It is possible that women
with altered arsenic metabolism may be more susceptible to arsenic’s cardiovascular effects and more likely to experience increases in blood pressure during pregnancy. Interestingly, in late pregnancy, a greater proportion of arsenic is excreted as MMA (Concha et al. 1998; Hopenhayn et al. 2003), possibly representing a detoxification mechanism. Although this pregnancy-related alteration in metabolism is not well understood, it is possible that this mechanism may in part account for the observed association between increased blood pressure in association with both PMI and SMI. Further study of the effect modification by arsenic metabolites is warranted, particularly at the lower levels of arsenic exposure found in US populations.

Urinary arsenic is considered to be a reliable short-term measure of arsenic exposure that appears to remain relatively consistent in adults, even during pregnancy (Ahmed et al. 2011; Gamble et al. 2006). In this study, we collected urine samples over a narrow gestational timeframe, during which concentrations were previously found not to vary (Gilbert-Diamond et al. 2011). In order to examine the trajectory of blood pressure over pregnancy, we used measurements beginning at 13 weeks gestation, thus some measurements were taken prior to urine sampling. However, prior studies suggest that total urinary arsenic levels remain relatively constant over pregnancy (Ahmed et al. 2011). However, our single exposure measurement may not be representative of typical exposure levels for all of the women in our study sample and that there may be variability in arsenic exposure levels that we were unable to account for in this study. Further, the Gamble et al. study was performed in adults and urinary arsenic stability may vary between non-pregnant and pregnant adults. While we do not collect multiple urine samples from participants, we collected maternal toenail samples prior to delivery, which approximately represent the previous 6 to 9 months of exposure. Among 334 women in our study with both prenatal urinary and toenail arsenic measurements, toenail arsenic was positively correlated with urinary arsenic.
measurements, \((r=0.33, p<0.001, \text{data not shown})\). Moreover, use of urine as an arsenic biomarker allows us to account for exposure from other sources, such as diet. However, nearly 1 in 8 individuals \((12.5\%)\) in this sample had water arsenic levels that exceeded the EPA maximum contaminant limit of \(10\mu g/L\), which likely represents the primary source of arsenic exposure among these individuals, work from our study area of New Hampshire has found that a variety of foods, including rice, can also significantly increase an individual’s arsenic exposure \((\text{Cottingham et al. 2013; Gilbert-Diamond et al. 2011})\).

Our study has some potential limitations. First, we used measurements of blood pressure at prenatal care visits, obtained from medical records. These measurements reflect the types of measurements and patterns that are obtained in routine clinical settings and although standard medical procedures were used, differences in staff and instrumentation may have introduced random variability into our measurements and blood pressure can fluctuate acutely, in relation to anxiety, recent exertion, and caffeine consumption, contributing to measurement error. Although we were not able to account for these factors in our models, we would not expect instrumentation to be related to exposure status and error in the precision of measurement techniques would likely bias our estimates toward the null. We also were unable to account for dietary factors (i.e. high sodium consumption, nutrient levels) that have the potential to impact blood pressure levels and due to sample size, we may have been limited in our ability to examine the impact of effect modifiers, such as age or BMI. Our study population of mothers tended to be well-educated, and primarily white, which may underrepresent different racial or socioeconomic groups that are at higher risk of gestational hypertension. Nonetheless, internal validity of the study is strengthened by the fact that we have multiple measurements for each woman over the course of pregnancy, detailed medical history and sociodemographic information from our
participants to include in our models. However, some women in our study were missing covariate information. We used multiple imputation methods to impute missing data and we cannot rule out the possibility that data were not missing completely at random. Further, our choice of mixed models helps to account for random variability. Longitudinal data analysis provides a sensitive tool for characterizing health outcomes that change gradually, such as blood pressure, and repeated measures can be a powerful way to identify small changes that can have a large impact at the population level (Farrington 1991). Blood pressure has a strong, continuous positive association with cardiovascular disease (Law et al. 2003; MacMahon et al. 1990; Sagie et al. 1993) and as SBP increases above 115mmHg, the risk of cardiovascular disease rises continuously (Vasan et al. 2001; Williams et al. 2008). As such, the changes observed here have the potential to impact maternal cardiovascular risks (Law et al. 2003).

It is becoming increasingly evident that pregnant women and the developing fetus are particularly vulnerable to environmental insults. Inorganic arsenic consumed both from drinking water and diet may contribute to overall arsenic burden in US pregnant women. While arsenic’s adverse cardiovascular effects have been investigated in adults, to our knowledge, our study is among the first to examine these impacts during pregnancy. As cardiovascular morbidity and mortality rise worldwide, the potential risk of later life cardiovascular diseases in mothers and children who are exposed to arsenic during pregnancy makes this a critical area of investigation.
References


Table 1. Selected characteristics of women enrolled in the New Hampshire Birth Cohort Study (n = 514), categorized by tertiles of pregnancy total urinary arsenic measurements. Data are n (%) or mean ± SD (range).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall U-As (0.35-288.5 µg/L) N = 514</th>
<th>First tertile U-As (0.35-2.54 µg/L) N = 171</th>
<th>Second tertile U-As (2.54-5.34 µg/L) N = 171</th>
<th>Third tertile U-As (5.34-288.5 µg/L) N = 172</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at enrollment, years</td>
<td>31.1 ± 4.9 (18.5-44.6)</td>
<td>30.7 ± 4.9 (19.3-44.4)</td>
<td>31.5 ± 4.9 (18.5-44.6)</td>
<td>31.2 ± 4.9 (19.1-43.4)</td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 11th grade</td>
<td>4 (0.9)</td>
<td>0 (0)</td>
<td>2 (1.4)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>High school graduate</td>
<td>43 (9.9)</td>
<td>21 (14.9)</td>
<td>13 (9.0)</td>
<td>9 (6.3)</td>
</tr>
<tr>
<td>Junior college, some college, technical school</td>
<td>94 (21.6)</td>
<td>28 (18.8)</td>
<td>30 (20.7)</td>
<td>36 (25.4)</td>
</tr>
<tr>
<td>College graduate</td>
<td>173 (39.7)</td>
<td>57 (38.3)</td>
<td>60 (41.8)</td>
<td>56 (39.4)</td>
</tr>
<tr>
<td>Post-graduate schooling</td>
<td>122 (28.0)</td>
<td>43 (28.9)</td>
<td>40 (27.6)</td>
<td>39 (27.5)</td>
</tr>
<tr>
<td>Missing</td>
<td>78</td>
<td>22</td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td>Relationship status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>377 (86.1)</td>
<td>128 (85.3)</td>
<td>130 (89.0)</td>
<td>119 (83.8)</td>
</tr>
<tr>
<td>Single</td>
<td>48 (11.0)</td>
<td>19 (12.7)</td>
<td>12 (8.2)</td>
<td>17 (12.0)</td>
</tr>
<tr>
<td>Divorced, widowed</td>
<td>13 (3.0)</td>
<td>3 (2.0)</td>
<td>4 (2.7)</td>
<td>6 (4.2)</td>
</tr>
<tr>
<td>Missing</td>
<td>76</td>
<td>21</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Pre-pregnancy BMI, kg/m²</td>
<td>25.1 ± 5.1 (17.6-48.3)</td>
<td>24.5 ± 4.5 (18.0-42.5)</td>
<td>25.2 ± 5.0 (17.6-45.7)</td>
<td>25.7 ± 5.8 (17.6-48.3)</td>
</tr>
<tr>
<td>Missing</td>
<td>77</td>
<td>27</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>197 (38.5)</td>
<td>70 (41.4)</td>
<td>67 (39.2)</td>
<td>60 (34.9)</td>
</tr>
<tr>
<td>1</td>
<td>200 (39.1)</td>
<td>65 (38.5)</td>
<td>64 (37.4)</td>
<td>71 (41.3)</td>
</tr>
<tr>
<td>2 or more</td>
<td>115 (22.5)</td>
<td>34 (20.1)</td>
<td>40 (23.4)</td>
<td>41 (23.8)</td>
</tr>
<tr>
<td>Missing</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Developed gestational hypertension</td>
<td>8 (1.6)</td>
<td>4 (2.3)</td>
<td>1 (0.6)</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>Developed gestational diabetes</td>
<td>36 (7.0)</td>
<td>11 (6.4)</td>
<td>15 (8.8)</td>
<td>10 (5.8)</td>
</tr>
<tr>
<td>Smoked during pregnancy</td>
<td>26 (5.8)</td>
<td>10 (5.9)</td>
<td>4 (2.3)</td>
<td>12 (7.0)</td>
</tr>
<tr>
<td>Missing</td>
<td>66</td>
<td>18</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>Well water arsenic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, µg/L</td>
<td>4.3 ± 11.0 (0.0-147.7)</td>
<td>2.2 ± 5.9 (0.0-58.0)</td>
<td>3.1 ± 8.2 (0.0-67.5)</td>
<td>7.7 ± 15.9 (0.0-147.7)*</td>
</tr>
<tr>
<td>Above 10 µg/L MCL</td>
<td>58 (12.5)</td>
<td>10 (5.8)</td>
<td>11 (6.4)</td>
<td>37 (21.5)</td>
</tr>
<tr>
<td>Missing</td>
<td>51</td>
<td>15</td>
<td>15</td>
<td>21</td>
</tr>
</tbody>
</table>

U-As: Urinary arsenic. Frequencies and means were compared by chi-square or one-way ANOVA, respectively. *Significantly different from tertile 1 (p<0.001)
Table 2. Relation between pregnancy urinary arsenic and changes in blood pressure (mmHg) per month over pregnancy among 514 women in the New Hampshire Birth Cohort Study.

<table>
<thead>
<tr>
<th>As Exposure Measure (per 5 µg/L)</th>
<th>Number of BP measurements</th>
<th>SBP $\beta_{12}$ (95% CI)$^a$</th>
<th>p-value$^b$</th>
<th>DBP $\beta_{12}$ (95% CI)$^a$</th>
<th>p-value$^b$</th>
<th>PP $\beta_{12}$ (95% CI)$^a$</th>
<th>p-value$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total As</td>
<td>5032</td>
<td>0.15 (0.02, 0.29)</td>
<td>p = 0.022</td>
<td>0.02 (-0.08, 0.12)</td>
<td>p = 0.73</td>
<td>0.14 (0.02, 0.25)</td>
<td>p = 0.021</td>
</tr>
<tr>
<td>MMA</td>
<td>5016</td>
<td>1.28 (-0.27, 2.83)</td>
<td>p = 0.11</td>
<td>-0.25 (-1.45, 0.96)</td>
<td>p = 0.69</td>
<td>1.54 (0.16, 2.92)</td>
<td>p = 0.028</td>
</tr>
<tr>
<td>DMA</td>
<td>5032</td>
<td>0.18 (0.02, 0.33)</td>
<td>p = 0.022</td>
<td>0.03 (-0.09, 0.14)</td>
<td>p = 0.67</td>
<td>0.15 (0.02, 0.29)</td>
<td>p = 0.027</td>
</tr>
<tr>
<td>iAs</td>
<td>5031</td>
<td>1.11 (-0.23, 2.44)</td>
<td>p = 0.10</td>
<td>-0.01 (-1.04, 1.03)</td>
<td>p = 0.98</td>
<td>1.18 (-0.01, 2.38)</td>
<td>p = 0.052</td>
</tr>
</tbody>
</table>

BP: blood pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure. $^a$Coefficient in relation to interaction between a 5 µg/L increase in total urinary arsenic, MMA, DMA or iAs and each month of gestation. Adjusted for age at enrollment, pre-pregnancy BMI, educational level, marital status, maternal smoking, parity, gestational diabetes, and number of blood pressure measurements per participant. $^b$P-values for $\beta_{12}$ effect estimates.
Table 3. Relation between pregnancy total urinary arsenic and changes in blood pressure (mmHg) over pregnancy among 514 women in the New Hampshire Birth Cohort Study, stratified by methylation indices.

<table>
<thead>
<tr>
<th>Primary Methylation Index</th>
<th>Number of BP measurements</th>
<th>SBP $\beta_{12}$ (95% CI)$^a$</th>
<th>DBP $\beta_{12}$ (95% CI)$^a$</th>
<th>PP $\beta_{12}$ (95% CI)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$p$-value$^b$</td>
<td>$p$-value$^b$</td>
<td>$p$-value$^b$</td>
</tr>
<tr>
<td>Low PMI</td>
<td>2535</td>
<td>0.06 (-0.17, 0.29) $p = 0.60$</td>
<td>-0.02 (-0.19, 0.14) $p = 0.76$</td>
<td>0.10 (-0.10, 0.29) $p = 0.33$</td>
</tr>
<tr>
<td>High PMI</td>
<td>2475</td>
<td>0.23 (0.07, 0.39) $p = 0.004$</td>
<td>0.05 (-0.09, 0.19) $p = 0.51$</td>
<td>0.18 (0.03, 0.34) $p = 0.021$</td>
</tr>
<tr>
<td><strong>P for interaction</strong>$^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Low SMI</strong></td>
<td>2464</td>
<td>0.05 (-0.15, 0.26) $p = 0.61$</td>
<td>-0.06 (-0.22, 0.10) $p = 0.47$</td>
<td>0.12 (-0.06, 0.31) $p = 0.19$</td>
</tr>
<tr>
<td>High SMI</td>
<td>2546</td>
<td>0.25 (0.07, 0.42) $p = 0.005$</td>
<td>0.09 (-0.05, 0.22) $p = 0.22$</td>
<td>0.16 (0.01, 0.31) $p = 0.040$</td>
</tr>
<tr>
<td><strong>P for interaction</strong>$^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BP: blood pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure, PMI: Primary Methylation Index (MMA/iAs), SMI: Secondary Methylation Index (DMA/MMA). $^a$Coefficient in relation to interaction between a $5\mu$g/L increase in total urinary arsenic and each month of gestation. Adjusted for age at enrollment, pre-pregnancy BMI, educational level, marital status, maternal smoking, parity, gestational diabetes, and number of blood pressure measurements per participant. $^b$P values for $\beta_{12}$ effect estimates. $^c$P for interaction, based on two-tailed tests of significance.
**Figure Legend**

**Figure 1.** Blood pressure measurements over pregnancy by gestational week. For each two-week period, all (a) systolic blood pressure, (b) diastolic blood pressure or (c) pulse pressure measurements during that time were averaged first individually for each woman and then averaged across all women and plotted. Vertical bars represent the 95% confidence intervals. Measurements prior to six weeks of gestation were excluded due to few available measurements.
Figure 1

A

Systolic Blood Pressure, mmHg

Gestational Week

B

Diastolic Blood Pressure, mmHg

Gestational Week

C

Pulse Pressure, mmHg

Gestational Week