



# ENVIRONMENTAL HEALTH PERSPECTIVES

<http://www.ehponline.org>

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

Shohreh F. Farzan, Yu Chen, Fen Wu, Jieying Jiang, Mengling  
Liu, Emily Baker, Susan A. Korrick, Margaret R. Karagas

<http://dx.doi.org/10.1289/ehp.1408472>

**Received: 25 March 2014**

**Accepted: 18 March 2015**

**Advance Publication: 20 March 2015**

This article will be available in its final, 508-conformant form 2–4 months after Advance Publication. If you need assistance accessing this article before then, please contact [ehp508@niehs.nih.gov](mailto:ehp508@niehs.nih.gov). Our staff will work with you to assess and meet your accessibility needs within 3 working days.



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

Shohreh F. Farzan<sup>1,2,3</sup>, Yu Chen<sup>3</sup>, Fen Wu<sup>3</sup>, Jieying Jiang<sup>3</sup>, Mengling Liu<sup>3</sup>, Emily Baker<sup>1,4</sup>, Susan A. Korrick<sup>5,6</sup>, and Margaret R. Karagas<sup>1,2</sup>

<sup>1</sup>Children's Environmental Health and Disease Prevention Research Center at Dartmouth, Hanover, New Hampshire, USA; <sup>2</sup>Department of Epidemiology, Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire, USA; <sup>3</sup>Department of Population Health, New York University School of Medicine, New York, New York, USA; <sup>4</sup>Department of Obstetrics and Gynecology, Dartmouth Hitchcock Medical Center, Lebanon, New Hampshire, USA;

<sup>5</sup>Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA; <sup>6</sup>Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA

**Address correspondence to** Margaret R. Karagas, Section of Biostatistics and Epidemiology, Geisel School of Medicine, One Medical Center Drive, 7927 Rubin, Lebanon, NH 03756 USA. Telephone: (603) 653-9010. E-mail: [margaret.r.karagas@dartmouth.edu](mailto:margaret.r.karagas@dartmouth.edu)

**Running title:** Arsenic exposure and gestational blood pressure

**Acknowledgments:** S.F. Farzan is supported by a NIEHS K99/R00 (K99ES024144), the NIEHS Superfund Research Program (P42ES007373) and a KC Donnelly Externship supplement. Y. Chen is supported by a NIH R01 (R01ES017541), the NYU EHS center (P30ES000260) and the Columbia University Superfund (P42ES010349). The Children's Center for Environmental Health and Disease Prevention Research at Dartmouth is supported by NIEHS and USEPA

grants (ES022832, RD83459901). The funding agencies that supported this work had no role in the planning, design, or execution of this study, nor any role in data analysis or manuscript preparation.

**Competing financial interests:** The authors have no competing financial interests.

## **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 ( $iAs^{III}$ ), arsenate ( $iAs^V$ ), 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  $\mu g/L$  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<sup>III</sup>+iAs<sup>V</sup>) 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:

$$BP_{ij} = [\beta_0 + \beta_1(\text{TIME})_{ij} + \beta_2As_{0j} + \beta_{12}As_{0j}(\text{TIME})_{ij} + \alpha^T Z_{0j}] + [\mu_{0j} + \mu_{1j}(\text{TIME})_{ij}] + r_{ij} \quad [1]$$

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  $j$ ;  $\text{TIME}$  is gestational month of BP measurement;  $\beta_1$  is the coefficient for the association between  $\text{TIME}$  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  $\mu_{1j}$  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,  $5\mu\text{g/L}$  ( $\sim 1$  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/m<sup>2</sup>).

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 5 $\mu$ g/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 5 $\mu$ g/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/m<sup>3</sup> increase in PM<sub>10</sub> 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 PM<sub>10</sub> and O<sub>3</sub> 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; Hoppenhayn 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, ( $r=0.33$ ,  $p<0.001$ , 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  $10\mu\text{g/L}$ , 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 (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

- Abhyankar LN, Jones MR, Guallar E, Navas-Acien A. 2012. Arsenic exposure and hypertension: A systematic review. *Environ Health Perspect* 120(4):494–500.
- Ahmed S, Mahabbat-E Khoda S, Rekha RS, Gardner RM, Ameer SS, Moore S, et al. 2011. Arsenic-associated oxidative stress, inflammation, and immune disruption in human placenta and cord blood. *Environ Health Perspect* 119(2):258–264.
- Allen VM, Joseph K, Murphy KE, Magee LA, Ohlsson A. 2004. The effect of hypertensive disorders in pregnancy on small for gestational age and stillbirth: A population based study. *BMC Pregnancy Childbirth* 4(1):17.
- Bakker R, Steegers EA, Mackenbach JP, Hofman A, Jaddoe VW. 2010. Maternal smoking and blood pressure in different trimesters of pregnancy: The Generation R study. *J Hypertens* 28(11):2210–2218.
- Bryson CL, Ioannou GN, Rulyak SJ, Critchlow C. 2003. Association between gestational diabetes and pregnancy-induced hypertension. *Am J Epidemiol* 158(12):1148–1153.
- Buchet JP, Lauwerys R, Roels H. 1981. Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. *Int Arch Occup Environ Health* 48(1):71–79.
- Burgess JL, Kurzius-Spencer M, O'rourke MK, Littau SR, Roberge J, Meza-Montenegro MM, et al. 2013. Environmental arsenic exposure and serum matrix metalloproteinase-9. *J Expo Sci Environ Epidemiol* 23(2):163–169.
- Carpenter MW. 2007. Gestational diabetes, pregnancy hypertension, and late vascular disease. *Diabetes Care* 30 Suppl 2:S246–250.
- Chen CJ, Hsueh YM, Lai MS, Shyu MP, Chen SY, Wu MM, et al. 1995. Increased prevalence of hypertension and long-term arsenic exposure. *Hypertension* 25(1):53–60.
- Chen Y, Factor-Litvak P, Howe GR, Graziano JH, Brandt-Rauf P, Parvez F, et al. 2007a. Arsenic exposure from drinking water, dietary intakes of B vitamins and folate, and risk of high blood pressure in Bangladesh: A population-based, cross-sectional study. *Am J Epidemiol* 165(5):541–552.
- Chen Y, Graziano JH, Parvez F, Liu M, Slavkovich V, Kalra T, et al. 2011. Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. *BMJ* 342:d2431.

- Chen Y, Santella RM, Kibriya MG, Wang Q, Kappil M, Verret WJ, et al. 2007b. Association between arsenic exposure from drinking water and plasma levels of soluble cell adhesion molecules. *Environ Health Perspect* 115(10):1415–1420.
- Chen Y, Wu F, Graziano JH, Parvez F, Liu M, Paul RR, et al. 2013. Arsenic exposure from drinking water, arsenic methylation capacity, and carotid intima-media thickness in Bangladesh. *Am J Epidemiol* 178(3):372–381.
- Churchill D, Perry IJ, Beevers DG. 1997. Ambulatory blood pressure in pregnancy and fetal growth. *Lancet* 349(9044):7–10.
- Concha G, Vogler G, Lezcano D, Nermell B, Vahter M. 1998. Exposure to inorganic arsenic metabolites during early human development. *Toxicol Sci* 44(2):185–190.
- Cottingham KL, Karimi R, Gruber JF, Zens MS, Sayarath V, Folt CL, et al. 2013. Diet and toenail arsenic concentrations in a New Hampshire population with arsenic-containing water. *Nut J* 12(1):149.
- Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY. 2010. *Williams Obstetrics*. 23rd Ed: McGraw-Hill.
- Davis MA, Li Z, Gilbert-Diamond D, Mackenzie TA, Cottingham KL, Jackson BP, et al. 2014. Infant toenails as a biomarker of in utero arsenic exposure. *J Expos Sci Environ Epidemiol* 24(5):467–473.
- Davis MA, Mackenzie TA, Cottingham KL, Gilbert-Diamond D, Punshon T, Karagas MR. 2012. Rice consumption and urinary arsenic concentrations in US children. *Environ Health Perspect* 120(10):1418–1424.
- Demidenko E. 2004. *Mixed models: Theory and application*. 1 Ed. New York, NY: Wiley-Interscience.
- Farrington DP. 1991. Longitudinal research strategies: Advantages, problems, and prospects. *J Am Acad Child Adolesc Psychiatry* 30(3):369–374.
- Farzan SF, Korrick S, Li Z, Enelow R, Gandolfi AJ, Madan J, et al. 2013. In utero arsenic exposure and infant infection in a United States cohort: A prospective study. *Environ Res* 126:24–30.
- Fukushima K, Morokuma S, Tsukimori K, Murata M, Wake N. 2012. Blood pressure measurements within the JNC7 pre-hypertensive range after 32 weeks of gestation are a risk factor for decreased fetal growth. *Hypertension Res* 35(1):128–130.

- Gallo LA, Denton KM, Moritz KM, Tare M, Parkington HC, Davies M, et al. 2012. Long-term alteration in maternal blood pressure and renal function after pregnancy in normal and growth-restricted rats. *Hypertension* 60(1):206–213.
- Gamble MV, Liu X, Ahsan H, Pilsner JR, Ilievski V, Slavkovich V, et al. 2006. Folate and arsenic metabolism: A double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. *Am J Clin Nutr* 84(5):1093–1101.
- Gilbert-Diamond D, Cottingham KL, Gruber JF, Punshon T, Sayarath V, Gandolfi AJ, et al. 2011. Rice consumption contributes to arsenic exposure in US women. *Proc Natl Acad Sci USA* 108(51):20656–20660.
- Henriques AC, Carvalho FH, Feitosa HN, Macena RH, Mota RM, Alencar JC. 2014. Endothelial dysfunction after pregnancy-induced hypertension. *Int J Gynaecol Obstet* 124 (3):230–4.
- Hopenhayn C, Huang B, Christian J, Peralta C, Ferreccio C, Atallah R, et al. 2003. Profile of urinary arsenic metabolites during pregnancy. *Environ Health Perspect* 111(16):1888–1891.
- Houston MC. 2007. The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infarction. *Altern Ther Health Med* 13(2):S128–33
- Hsieh YC, Lien LM, Chung WT, Hsieh FI, Hsieh PF, Wu MM, et al. 2011. Significantly increased risk of carotid atherosclerosis with arsenic exposure and polymorphisms in arsenic metabolism genes. *Environ Res* 111(6):804–810.
- Huang YL, Hsueh YM, Huang YK, Yip PK, Yang MH, Chen CJ. 2009. Urinary arsenic methylation capability and carotid atherosclerosis risk in subjects living in arsenicosis-hyperendemic areas in southwestern Taiwan. *Sci Total Environ* 407(8):2608–2614.
- Irgens HU, Reisaeter L, Irgens LM, Lie RT. 2001. Long term mortality of mothers and fathers after pre-eclampsia: Population based cohort study. *BMJ* 323(7323):1213–1217.
- Jedrychowski WA, Perera FP, Maugeri U, Spengler J, Mroz E, Flak E, et al. 2012. Prohypertensive effect of gestational personal exposure to fine particulate matter: Prospective cohort study in non-smoking and non-obese pregnant women. *Cardiovasc Toxicol* 12(3):216–225.
- Karagas MR, Stukel TA, Tosteson TD. 2002. Assessment of cancer risk and environmental levels of arsenic in New Hampshire. *Int J Hyg Environ Health* 205(1-2):85–94.

- Kennedy DA, Woodland C, Koren G. 2012. Lead exposure, gestational hypertension and pre-eclampsia: a systematic review of cause and effect. *J Obstet Gynaecol* 32(6):512–517.
- Kwok RK, Mendola P, Liu ZY, Savitz DA, Heiss G, Ling HL, et al. 2007. Drinking water arsenic exposure and blood pressure in healthy women of reproductive age in Inner Mongolia, China. *Toxicol Appl Pharmacol* 222(3):337–343.
- Larsen EH, Pritzl G, Hansen SH. 1993. Speciation of 8 arsenic compounds in human urine by high-performance liquid-chromatography with inductively-coupled plasma-mass spectrometric detection using antimonate for internal chromatographic standardization. *J Anal At Spectrom* 8(4):557–563.
- Law M, Wald N, Morris J. 2003. Lowering blood pressure to prevent myocardial infarction and stroke: A new preventive strategy. *Health Technol Assess* 7(31):1–94.
- Le XC, Lu XF, Ma MS, Cullen WR, Aposhian HV, Zheng BS. 2000. Speciation of key arsenic metabolic intermediates in human urine. *Anal Chem* 72(21):5172–5177.
- Lee PC, Talbott EO, Roberts JM, Catov JM, Bilonick RA, Stone RA, et al. 2012. Ambient air pollution exposure and blood pressure changes during pregnancy. *Environ Res* 117:46–53.
- Little RJA, Rubin DB. 2002. Statistical analysis with missing data. 2nd Ed. New York, NY: Wiley-Interscience.
- MacMahon S, Peto R, Collins R, Godwin J, MacMahon S, Cutler J, et al. 1990. Blood pressure, stroke, and coronary heart disease: Part 1, prolonged differences in blood pressure: Prospective observational studies corrected for the regression dilution bias. *Lancet* 335(8692):765–774.
- Magnussen EB, Vatten LJ, Smith GD, Romundstad PR. 2009. Hypertensive disorders in pregnancy and subsequently measured cardiovascular risk factors. *Obstet Gynecol* 114(5):961–970.
- Matkin CC, Britton J, Samuels S, Eskenazi B. 1999. Smoking and blood pressure patterns in normotensive pregnant women. *Paediatr Perinat Epidemiol* 13(1):22–34.
- Miller RS, Thompson ML, Williams MA. 2007. Trimester-specific blood pressure levels in relation to maternal pre-pregnancy body mass index. *Paediatr Perinat Epidemiol* 21(6):487–494.
- Moon K, Guallar E, Navas-Acien A. 2012. Arsenic exposure and cardiovascular disease: An updated systematic review. *Curr Atheroscler Rep* 14(6):542–555.

- Moon KA, Guallar E, Umans JG, Devereux RB, Best LG, Francesconi KA, et al. 2013. Association between exposure to low to moderate arsenic levels and incident cardiovascular disease: A prospective cohort study. *Ann Intern Med* 159(10):649–659.
- Nachman KE, Baron PA, Raber G, Francesconi KA, Navas-Acien A, Love DC. 2013. Roxarsone, inorganic arsenic, and other arsenic species in chicken: A US-based market basket sample. *Environ Health Perspect* 121(7):818–824.
- NRC (National Research Council). 2014. Critical aspects of EPA's IRIS assessment of inorganic arsenic: Interim report. Washington, DC: National Research Council. Available: [http://www.nap.edu/openbook.php?record\\_id=18594](http://www.nap.edu/openbook.php?record_id=18594). [Accessed 2 March 2015]
- Navas-Acien A, Nachman KE. 2013. Public health responses to arsenic in rice and other foods. *JAMA Intern Med* 173(15):1395–1396.
- Nisell H, Lintu H, Lunell NO, Mollerstrom G, Pettersson E. 1995. Blood pressure and renal function seven years after pregnancy complicated by hypertension. *Br J Obstet Gynaecol* 102(11):876–881.
- Ray JG, Burrows RF, Burrows EA, Vermeulen MJ. 2001. MOS HIP: McMaster outcome study of hypertension in pregnancy. *Early Hum Dev* 64(2):129–143.
- Roberts CL, Algert CS, Morris JM, Ford JB, Henderson-Smart DJ. 2005. Hypertensive disorders in pregnancy: A population-based study. *Med J Aust* 182(7):332–335.
- Sagie A, Larson MG, Levy D. 1993. The natural history of borderline isolated systolic hypertension. *N Engl J Med* 329(26):1912–1917.
- Skjaerven R, Wilcox AJ, Klungsoyr K, Irgens LM, Vikse BE, Vatten LJ, et al. 2012. Cardiovascular mortality after pre-eclampsia in one child mothers: Prospective, population based cohort study. *BMJ* 345:e7677.
- States JC, Srivastava S, Chen Y, Barchowsky A. 2009. Arsenic and cardiovascular disease. *Toxicol Sci* 107(2):312–323.
- Thompson ML, Miller RS, Williams MA. 2007. Construction and characterisation of a longitudinal clinical blood pressure database for epidemiological studies of hypertension in pregnancy. *Paediatr Perinat Epidemiol* 21(6):477–486.
- Tseng CH. 2009. A review on environmental factors regulating arsenic methylation in humans. *Toxicol Appl Pharmacol* 235(3):338–350.

- U.S. EPA (U.S. Environmental Protection Agency). 2000. Arsenic occurrence in public drinking water supplies. Available: <http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=901O0P00.txt>. [accessed 2 March 2015].
- Vahter M. 1999. Methylation of inorganic arsenic in different mammalian species and population groups. *Science Progress* 82 (Pt 1):69–88.
- van den Hooven EH, De Kluizenaar Y, Pierik FH, Hofman A, Van Ratingen SW, Zandveld PY, et al. 2011. Air pollution, blood pressure, and the risk of hypertensive complications during pregnancy: The Generation R study. *Hypertension* 57(3):406–412.
- Vasan RS, Larson MG, Leip EP, Evans JC, O'donnell CJ, Kannel WB, et al. 2001. Impact of high-normal blood pressure on the risk of cardiovascular disease. *N Engl J Med* 345(18):1291–1297.
- Vikse BE, Irgens LM, Leivestad T, Skjaerven R, Iversen BM. 2008. Preeclampsia and the risk of end-stage renal disease. *N Engl J Med* 359(8):800–809.
- Wang IK, Muo CH, Chang YC, Liang CC, Chang CT, Lin SY, et al. 2013. Association between hypertensive disorders during pregnancy and end-stage renal disease: A population-based study. *Can Med Assoc J* 185(3):207–213.
- Wang S-L, Chang F-H, Liou S-H, Wang H-J, Li W-F, Hsieh DPH. 2007. Inorganic arsenic exposure and its relation to metabolic syndrome in an industrial area of Taiwan. *Environ Int* 33(6):805–811.
- Wei HY, Brockhoff-Schwegel CA, Creed JT. 2001. A comparison of urinary arsenic speciation via direct nebulization and on-line photo-oxidation-hydride generation with IC separation and ICP-MS detection. *J Anal At Spectrom* 16(1):12–19.
- Weissgerber TL, Turner ST, Bailey KR, Mosley TH, Jr., Kardia SL, Wiste HJ, et al. 2013. Hypertension in pregnancy is a risk factor for peripheral arterial disease decades after pregnancy. *Atherosclerosis* 229(1):212–216.
- Wells EM, Goldman LR, Jarrett JM, Apelberg BJ, Herbstman JB, Caldwell KL, et al. 2012. Selenium and maternal blood pressure during childbirth. *J Expos Sci Environ Epidemiol* 22:191–197.
- Williams B, Lindholm LH, Sever P. 2008. Systolic pressure is all that matters. *Lancet* 371(9631):2219–2221.

- Wilson BJ, Watson MS, Prescott GJ, Sunderland S, Campbell DM, Hannaford P, et al. 2003. Hypertensive diseases of pregnancy and risk of hypertension and stroke in later life: Results from cohort study. *BMJ* 326(7394):845.
- WHO (World Health Organization). 2008. The global burden of disease: 2004 update. Geneva, Switzerland: World Health Organization. Available: [http://www.who.int/healthinfo/global\\_burden\\_disease/2004\\_report\\_update/en/](http://www.who.int/healthinfo/global_burden_disease/2004_report_update/en/). [Accessed 2 March 2015].
- Wu F, Jasmine F, Kibriya MG, Liu M, Wojcik O, Parvez F, et al. 2012. Association between arsenic exposure from drinking water and plasma levels of cardiovascular markers. *Am J Epidemiol* 175(12):1252–1261.
- Wu MM, Kuo TL, Hwang YH, Chen CJ. 1989. Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. *Am J Epidemiol* 130(6):1123–1132.
- Xiong X, Fraser WD. 2004. Impact of pregnancy-induced hypertension on birthweight by gestational age. *Paediatr Perinat Epidemiol* 18(3):186–191.
- Yazbeck C, Thiebaugeorges O, Moreau T, Goua V, Debotte G, Sahuquillo J, et al. 2009. Maternal blood lead levels and the risk of pregnancy-induced hypertension: The Eden cohort study. *Environ Health Perspect* 117(10):1526–1530.
- Yoder SR, Thornburg LL, Bisognano JD. 2009. Hypertension in pregnancy and women of childbearing age. *Am J Med* 122(10):890–895.
- Zhang J, Villar J, Sun W, Merialdi M, Abdel-Aleem H, Mathai M, et al. 2007. Blood pressure dynamics during pregnancy and spontaneous preterm birth. *Am J Obstet Gynecol* 197(2): 162.e161–166.

**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).

Variable	Overall U-As (0.35-288.5 µg/L) N = 514	First tertile U-As (0.35-2.54 µg/L) N = 171	Second tertile U-As (2.54-5.34 µg/L) N = 171	Third tertile U-As (5.34-288.5 µg/L) N = 172
Age at enrollment, years	31.1 ± 4.9 (18.5-44.6)	30.7 ± 4.9 (19.3-44.4)	31.5 ± 4.9 (18.5-44.6)	31.2 ± 4.9 (19.1-43.4)
Level of education				
Less than 11 <sup>th</sup> grade	4 (0.9)	0 (0)	2 (1.4)	2 (1.4)
High school graduate	43 (9.9)	21 (14.9)	13 (9.0)	9 (6.3)
Junior college, some college, technical school	94 (21.6)	28 (18.8)	30 (20.7)	36 (25.4)
College graduate	173 (39.7)	57 (38.3)	60 (41.8)	56 (39.4)
Post-graduate schooling	122 (28.0)	43 (28.9)	40 (27.6)	39 (27.5)
Missing	78	22	26	30
Relationship status				
Married	377 (86.1)	128 (85.3)	130 (89.0)	119 (83.8)
Single	48 (11.0)	19 (12.7)	12 (8.2)	17 (12.0)
Divorced, widowed	13 (3.0)	3 (2.0)	4 (2.7)	6 (4.2)
Missing	76	21	25	30
Pre-pregnancy BMI, kg/m <sup>2</sup>	25.1 ± 5.1 (17.6-48.3)	24.5 ± 4.5 (18.0-42.5)	25.2 ± 5.0 (17.6-45.7)	25.7 ± 5.8 (17.6-48.3)
Missing	77	21	25	31
Parity				
0	197 (38.5)	70 (41.4)	67 (39.2)	60 (34.9)
1	200 (39.1)	65 (38.5)	64 (37.4)	71 (41.3)
2 or more	115 (22.5)	34 (20.1)	40 (23.4)	41 (23.8)
Missing	2	2	0	0
Developed gestational hypertension	8 (1.6)	4 (2.3)	1 (0.6)	3 (1.7)
Developed gestational diabetes	36 (7.0)	11 (6.4)	15 (8.8)	10 (5.8)
Smoked during pregnancy	26 (5.8)	10 (2.9)	4 (2.3)	12 (7.0)
Missing	66	18	22	26
Well water arsenic				
Mean, µg/L	4.3 ± 11.0 (0.0-147.7)	2.2 ± 5.9 (0.0-58.0)	3.1 ± 8.2 (0.0-67.5)	7.7 ± 15.9 (0.0-147.7)*
Above 10 µg/L MCL	58 (12.5)	10 (5.8)	11 (6.4)	37 (21.5)
Missing	51	15	15	21

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.

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

BP: blood pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure. <sup>a</sup>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. <sup>b</sup>*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.

Primary Methylation Index	Number of BP measurements	SBP $\beta_{12}$ (95% CI) <sup>a</sup> <i>p-value</i> <sup>b</sup>	DBP $\beta_{12}$ (95% CI) <sup>a</sup> <i>p-value</i> <sup>b</sup>	PP $\beta_{12}$ (95% CI) <sup>a</sup> <i>p-value</i> <sup>b</sup>
Low PMI	2535	0.06 (-0.17, 0.29) <i>p</i> = 0.60	-0.02 (-0.19, 0.14) <i>p</i> = 0.76	0.10 (-0.10, 0.29) <i>p</i> = 0.33
High PMI	2475	0.23 (0.07, 0.39) <i>p</i> = 0.004	0.05 (-0.09, 0.19) <i>p</i> = 0.51	0.18 (0.03, 0.34) <i>p</i> = 0.021
		<i>P for interaction</i> <sup>c</sup> = 0.21	<i>P for interaction</i> <sup>c</sup> = 0.52	<i>P for interaction</i> <sup>c</sup> = 0.47
Secondary Methylation Index				
Low SMI	2464	0.05 (-0.15, 0.26) <i>p</i> = 0.61	-0.06 (-0.22, 0.10) <i>p</i> = 0.47	0.12 (-0.06, 0.31) <i>p</i> = 0.19
High SMI	2546	0.25 (0.07, 0.42) <i>p</i> = 0.005	0.09 (-0.05, 0.22) <i>p</i> = 0.22	0.16 (0.01, 0.31) <i>p</i> = 0.040
		<i>P for interaction</i> <sup>c</sup> = 0.17	<i>P for interaction</i> <sup>c</sup> = 0.17	<i>P for interaction</i> <sup>c</sup> = 0.74

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). <sup>a</sup>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. <sup>b</sup>*P* values for  $\beta_{12}$  effect estimates. <sup>c</sup>*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

