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A Pilot Study: The importance of inter-individual differences in inorganic arsenic metabolism for birth weight outcome

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Abstract

Inorganic arsenic (iAs) exposure is detrimental to birth outcome. We lack information regarding the potential for iAs metabolism to affect fetal growth. Our pilot study evaluated postpartum Romanian women with known birth weight outcome for differences in iAs metabolism. Subjects were chronically exposed to low-to-moderate drinking water iAs. We analyzed well water, arsenic metabolites in urine, and toenail arsenic. Urine iAs and metabolites, toenail iAs, and secondary methylation efficiency increased as an effect of exposure ($p<0.001$). Urine iAs and metabolites showed a significant interaction effect between exposure and birth weight. Moderately exposed women with low compared to normal birth weight outcome had greater metabolite excretion ($p<0.03$); 67% with low compared to 10% with normal birth weight outcome presented urine iAs >9μg/L ($p=0.019$). Metabolic partitioning of iAs toward excretion may impair fetal growth. Prospective studies on iAs excretion before and during pregnancy may provide a biomarker for poor fetal growth risk.

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Conflict of Interest
The authors declare no conflicts of interest.

Keywords

arsenic; birth weight; methylation ratio; Romania; biomarker

1. Introduction

The primary focus of existing inorganic arsenic (iAs) research in the field of reproductive health is the effect of exposure magnitude on fetal growth reduction (Hopenhayn et al., 2003a; Huyck et al., 2007; Yang et al., 2003), as well as other detrimental birth outcomes, *e.g.*, spontaneous abortion, and stillbirth (Ahmad et al., 2001; Rahman et al., 2007). In contrast to exposure-related information, we know little about underlying, background patterns of iAs metabolic handling in women that may detrimentally influence birth weight outcome. The role of inter-individual differences in iAs metabolism when considering birth weight outcome within a given exposure area could be reflected in patterns of underlying methylation efficiency, but also in patterns of pathway partitioning between excretion and retention. These potential underlying inter-individual differences in iAs metabolism may be especially important in regions of the world with low-to-moderate iAs exposure through drinking water.

IAs is metabolized via methylation in the liver to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Vahter, 2002). Determination of both the concentration and the proportional distribution of the urinary arsenic metabolites provide a measurement of the methylation efficiency of the human body (Chen et al., 2003a; Chen et al., 2003b). Previous research has documented that methylation efficiency increases during pregnancy (Vahter, 2009). Existing evidence suggests while there is a higher percentage of urine excreted as DMA versus MMA during pregnancy, there is apparent stability in the amount of urine iAs excreted (Hopenhayn et al., 2003b). Moreover, there is apparent stability in urine iAs based on prospective population data in both men and women (Kile et al., 2009). In contrast, inter-individual variability in urine iAs concentrations is appreciated to be relatively high. Inter-individual variability is thought to be dependant at least in part on genetic determinants of iAs methylation and excretion, even when exposure is equivalent (Concha et al., 2002; Tseng, 2009). Identifying a distinct underlying, metabolic pattern for iAs handling associated with low birth weight outcome in a given exposure region could better inform strategies to modify this risk.

Taken together, these data highlight that in addition to methylation efficiency, understanding the underlying metabolic partitioning patterns of iAs either toward excretion in urine or retention under conditions of low-to-moderate exposure is likely important in women. For example, when iAs and its metabolites circulate in high concentrations the fetus can be impacted directly, evidenced by the ready passage of all iAs metabolites through the placenta and bioaccumulation of iAs in the developing fetus (Concha et al., 1998; Hall et al., 2007). Besides this direct effect, there is the potential for a primary indirect effect of iAs due to its potential burden on one-carbon metabolism by diverting methyl groups away from growth processes (Gamble et al., 2006; Vahter, 2009). To that end, the increased burden on methylation pathways during pregnancy has been demonstrated to increase homocysteine levels, a risk factor for adverse pregnancy outcomes (Vahter, 2009). While we have a basic understanding of the consequence of magnitude of exposure to iAs on health, we do not know if normally high partitioning of ingested iAs toward methylation coupled with pregnancy, or other health outcome, places an identifiable extra burden on one-carbon metabolism pathways.

The World Health Organization, European Environment Agency, and the United States Environmental Protection Agency guidelines for arsenic in drinking water set a limit of 10 μg/l. The Arsenic Health Risk Assessment and Molecular Epidemiology (ASHRAM) measured drinking water arsenic exposure in the Western Romanian county of Arad between 0.1 and 196.0 μg/l (Hough et al., 2010; Leonardi et al., 2012). Recent data for infant mortality and prevalence of low birth weight indicate higher values in Romania than for the European Union (EU): mortality of 9.79 per 1,000 live births compared to the average EU value of 4.18 per 1,000 live births, and low birth weight of 8.03% compared to the EU value of 7.29% (HFA-DB, 2012). These data bring into question the potential role of low-to-moderate iAs exposure as a modifiable risk for fetal health.

Our pilot, metabolic study examined women with known birth weight outcome as a first step to increasing understanding of the importance of inter-individual differences in iAs metabolism in healthy women residing in low-to-moderate areas of endemic iAs in drinking water. The combination of previously identified, and well characterized stable regions of low-to-moderate iAs exposure through drinking water, and previously established clinic and data collection networks due to the ASHRAM study, as well as a stable resident population, made western Romania an advantageous study location for this pilot study (Gurzau and Gurzau, 2001; Hough et al., 2010; Leonardi et al., 2012; Lindberg et al., 2006).

2. Materials and Methods

2.1. Study population

This study was conducted in rural regions of Arad County in western Romania. Rural Arad County had 1,870 total births 2010 (INS, 2012a, b). Arad County contained 270 villages in 2010 (INS, 2012a). The study regions within Arad were identified by the multi-country ASHRAM study as having low-to-moderate levels of iAs in drinking water. IAs exposure through drinking water in Arad has been well described (Gurzau and Gurzau, 2001; Hough et al., 2010; Leonardi et al., 2012; Lindberg et al., 2006). Participants were recruited from four villages with low-to-moderate levels of arsenic exposure (exposed, ≥10 μg/L) and two villages with very low levels of arsenic exposure (unexposed, <1 μg/L). The villages had similar occupational, dietary, and lifestyle profiles. None of the participants in this metabolic study were part of the ASHRAM study.

For this pilot study, participants included forty-two healthy women between the ages of 18 and 36 at the time of their last pregnancy (mean 3.3±2.9 years prior to the study). Participants had either normal (>2500 g) or low (≤2500 g) birth weight pregnancy outcomes and were from both the exposed (Pilu, Varşand, Şepreuş, Apateu) and unexposed (Păuliş, Ghioroc) villages. Each of these rural villages was served by only one primary care center, which supplied all health care including pre-natal care. Pilu and Varşand are neighboring villages served by the same primary care center. Primary care physicians provided the advertising and recruitment for the study. The investigators identified an equal number of qualifying volunteer participants for all sub groups based on order of call-back. Participants included women who had given birth between 2000 and 2010 and had a long-standing residence (at least 18 years) in the locality to ensure long-term, stable exposure levels to iAs in drinking water and other environmental conditions before, during, and after pregnancy. Eligible pregnancies were single-birth, full-term (40-42 weeks calculated from the first date of the last menstruation and recorded in the woman's medical file by her doctor), and free of health complications. Four participants were excluded from the study – two did not arrive at the initial study visit, one had given birth to twins, and one was determined to have lived in the locality for only five years. There were no differences in non-participation in exposed versus unexposed villages. Thus, 38 participants were ultimately included in the study, 18 with low birth weight outcomes and 20 with normal birth weight outcomes.

Participants completed an initial study visit consisting of an interview, height and weight measurements, and the collection of a urine sample. A modified version of the validated ASHRAM questionnaire to establish health history, lifestyle indicators, water use patterns, current and previous residences, and occupational information was administered to participants by a trained interviewer (Hough et al., 2010). The questionnaire used in this study was consistent with ASHRAM questionnaire, however questions on sun exposure were removed and information on previous pregnancies, pregnancy outcomes, and behavior during pregnancy, including the use of prenatal vitamins, smoking, and alcohol consumption, was collected. A second follow-up visit was conducted for toenail sample collection. At this visit, two participants were not available. All study participants gave informed consent, and the protocol was approved by the institutional review boards of the regional public health authority of Arad County in Romania and Yale University School of Medicine. The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. Water analysis

Individual exposure assessment was conducted by collecting water samples from the current and past, when possible, main drinking water sources for each participant. Samples were collected and analyzed by standard methods using atomic absorption spectrometry (Varian 110, Palo Alto, CA) with vapor generation system VGA 77 (Lindberg et al., 2006). Arsenic measurements that fell below the detection limit were imputed as the detection limit of 1.0 μg/L. Exposure values were assigned to each participant using the average arsenic concentration in all main drinking water sources identified by the participant during the interview, methodology previously established by the ASHRAM study (Hough et al., 2010). The estimated total ingested arsenic dose from drinking water is defined as dose = water iAs concentration * liters of groundwater consumed per day. In this calculation, water consumption includes drinking water and tap water used to make hot or cold drinks, but does not account for water used in cooking. The selected biomarkers, urinary arsenic and iAs concentration of toenail samples, reflect intake of iAs from all sources of exposure including diet.

2.3. Urine analysis

Urine samples for all women were collected as spot urine samples at the time of the study. Dimethylarsinic acid (DMA^V), monomethylarsonic acid (MMA^V), and total inorganic arsenic (iAs) were separated with high performance liquid chromatography (Agilent 1100) and determined with inductively coupled plasma mass spectrometry (Agilent 7700cx Agilent Technologies, Waldbronn, Germany). Method details can be found in Scheer et al. 2012 (Scheer et al., 2012). Additionally, a portion of the urine was oxidized with H_2O_2 to convert any trivalent- and thio-arsenicals to their pentavalent and/or oxygenated forms. Arsenic concentrations were adjusted for dilution variation using average specific gravity in the sample population. Arsenic measurements that fell below the detection limit were imputed as the detection limit of 0.07 μg/L. The primary methylation index was calculated as the ratio of MMA/iAs and the secondary methylation index as the ratio of DMA/MMA.

2.4. Toenail analysis

Toenail iAs concentration is considered a stable biomarker of integrated iAs exposure over the previous 9-12 months (Karagas et al., 2000; Kile et al., 2007; Marchiset-Ferlay et al., 2012). IAs deposited in the nail matrix is not re-circulated in the body and is used as an exposure biomarker. Following analysis of the water and urine data, additional data were desired to obtain a more complete picture of the participants' As storage profiles. Thus, toenail samples were collected from each participant. The primary care physicians collected participant toenail clippings from all 10 toenails.

Sample pre-treatment—Before washing the nail samples, any visible dirt on the surface of nails was removed manually. The nails were rinsed five times with distilled water and then soaked in acetone for 30 minutes and rinsed again five times with distilled water. The samples were kept in labeled vials, oven dried over night at 50-60°C and then desiccated for 2 hours.

Acid digestion—The digestion was made in a microwave digestion system MARS 5 at 600 W in Teflon digestion vessels (XP 1500 plus). The dry sample was weighed and transferred into a digestion vessel where HNO_3 and HCl were added. The samples were kept in the exhausting fume hood for 1 hour and mixed periodically, before the microwave digestion.

HG-AAS analysis—After cooling, each sample was transferred to a 15 ml flask. Samples were diluted with distilled water. The samples were analyzed by HG-AAS ZENNIT 700P. The detection limit was 0.07 μg/L for liquid sample.

2.5. Statistical analysis

Data were analyzed using SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA). Study population characteristics, drinking water arsenic exposure, and urinary arsenic metabolites were compared between exposure groups and tested for significance (α=0.05) using the chi-square test, Fisher's exact test, and student t-test where appropriate. The arsenic metabolite data were analyzed for exposure and birth weight status using a multivariate analysis of variance (ANOVA) with log-transformed data. All metabolite concentrations were adjusted for specific gravity of the urine. Partial correlation analysis provided summary data for birth weight status, while controlling for BMI, age, and smoking status.

3. Results

Demographic data are presented in Table 1 for normal and low birth weight outcomes in exposed and unexposed locations. The women within each location were not significantly different in terms of age, BMI, sex of child, income, blood glucose, or number of prior pregnancies. Reported behaviors during and after pregnancy (*e.g.*, alcohol consumption, smoking, and prenatal vitamin use) were also not significantly different between groups. The only exception was that exposed normal birth weight participants reported significantly more years of education (10.6±2.4) than the exposed low birth weight participants (6.6±3.8) (p=0.02).

As expected, drinking water exposure as measured by concentration of iAs in a participant's primary drinking water source or average of multiple sources, was significantly different between the exposed (54.4±27.0 μg/l) and unexposed (1.1±0.1 μg/l) participants (p<0.01). In contrast, this measure of exposure did not vary between exposed women based on birth weight outcome (low birth weight: 56.9±24.7 μg/l, normal birth weight: 52.2±30.0 μg/l, p=0.71), or between unexposed women based on birth weight outcome (low birth weight: 1.1±0.1 μg/l, normal birth weight: 1.1±0.2 μg/l, p=0.91).

Measures of individual arsenic exposure (dose), primary and secondary methylation efficiency, as well as toenail arsenic (index of prolonged exposure) are presented in Figure 1. Main effects of exposure were observed for all parameters (p<0.05), except the primary methylation index. However, there were neither significant main effects of birth weight nor significant interaction effects for these variables. The total ingested arsenic dose from drinking water showed no significant difference between the unexposed women with low birth weight outcome (1.5±0.6 μg/day) and normal birth weight outcome (0.8±0.8 μg/day)

(p=0.07). Importantly, there was a similar finding for the exposed women. The calculated total iAs dose was not significantly different between low birth weight outcome (68.6±33.6 μg/day) and normal birth weight outcome (64.4±40.9 μg/day) (p=0.81).

The multivariate ANOVA was performed using exposure and birth weight groups as independent variables, and urinary arsenic metabolite concentrations as interrelated dependent variables. As expected, this analysis revealed significance of all factors as a main effect of exposure (all factors $p<0.001$). Unexpected, were the significant interaction effects between exposure and birth weight for all urine metabolite variables: DMA ($p=0.015$), MMA ($p=0.018$), and iAs ($p=0.020$) that favored trafficking toward excretion for the exposed, low birth weight outcome group (see Figure 2A-C). In contrast, when relative percentages of the metabolites were used as the dependent variables, the analysis showed neither significant birth weight effects nor significant interaction effects of birth weight with exposure. The only significant increases based on relative data were for the main effect of exposure for MMA(%) ($p<0.001$) and iAs(%) ($p=0.043$) (see Figure 2D-F).

In absolute terms, our pilot study supports the likelihood of a cut-point for urine iAs concentrations (Figure 3A). In exposed women, 67% of the low birth weight group, as compared to only 10% in the normal birth weight group ($p=0.019$) had urinary iAs concentrations greater than 9.0 μg/L (Figure 3B). In fact, none of the exposed women with normal birth weight infants had a urine iAs value ≥10 μg/L. Therefore, the exposed, low birth weight group exhibited an underlying metabolic partitioning toward methylation and excretion, which increased the likelihood of an elevated concentration of iAs in the urine.

Elevated partitioning toward excretion in women with low birth weight outcome is also emphasized by examining the partial correlation coefficients based on the two birth weight classifications (with all exposed and unexposed women included) (Table 2). Controlling for BMI, age, and smoking status, the data show the expected total significant correlation of drinking water iAs concentration and ingested dose ($r > 0.96$, $p<0.001$). In fact, all metabolite parameters (urine and toenail) for both birth weight groups show significant associations with exposure level (r values > 0.6, $p<0.05$). In contrast, while the low birth weight classification group shows significant associations of iAs with the methylation endpoints in the urine (all r values > 0.8, $p<0.001$), *none of these* comparisons are significant for the normal birth weight classification group. Note that the significant exposure-metabolite associations were not observed if relative (%) values replaced the absolute concentration values in the analysis.

4. Discussion

To the best of our knowledge, this pilot metabolic study provides the first evidence concerning the importance of inter-individual differences in underlying, non-pregnant iAs metabolism for birth weight outcome. Our findings were from women with long-standing residence in a region of Romania with well established and globally common low-to-moderate iAs exposure levels in drinking water. Underlying methylation efficiency was not distinct for birth weight outcome. In contrast, we found a significant interactive effect for iAs metabolic partitioning. Higher absolute levels of iAs and urinary arsenic metabolites were excreted in urine of the exposed women with low birth weight infants compared to those with normal birth weight infants. Moreover, these pilot data provide a preliminary estimate of iAs in urine >9.0 μg/L as a potential novel biomarker to indicate risk of poor fetal growth.

Several pharmacokinetic models have studied the deposition, methylation, and excretion of iAs in humans and in animals (El-Masri and Kenyon, 2008; Kenyon et al., 2008; Kitchin et

al., 1999). Much is known, therefore, regarding rate limiting steps, expected ratios of metabolites and how genetics, nutrition, exposure level, and other environmental co-exposures influence methylation of iAs (Tseng, 2009). Our data revealed the expected percentages of iAs metabolites in urine, but these relative values did not distinguish the low birth weight outcomes.

Previous research also shows arsenic exposure generally results in reduced overall methylation efficiency (Del Razo et al., 1997; Hopenhayn-Rich et al., 1996a; Kurttio et al., 1998). Our findings were in accord with respect to exposure; however, the methylation efficiency *per se* was not observed to be distinct *within* exposure groups. The implication for this finding is important because methylation efficiency is not stable during pregnancy (Concha et al., 1998; Gardner et al., 2011; Hopenhayn et al., 2003b), and thus would present a moving target unsuitable for guidelines regarding health risk for poor fetal growth where elevated endemic iAs in drinking water is a concern. Our data do not support the use of methylation efficiency as an underlying, background indicator for birth weight outcome at low-to-moderate exposure levels of iAs in drinking water.

In contrast to the similar background methylation efficiencies in women differing by birth weight outcome, we show the greater amount of iAs partitioned toward excretion characterized the exposed women with low birth weight outcomes. This interpretation was strengthened by the correlation analysis performed in all women based on birth weight status. Our data in low-to-moderate exposure conditions remain in accord with the theory that higher absolute concentrations of iAs and MMA are likely detrimental to health (Smith and Steinmaus, 2009).

Important to our assessment and interpretation of underlying inter-individual differences in iAs metabolic partitioning is the stability of iAs concentrations in urine. Limited research during pregnancy demonstrates that absolute urinary iAs concentrations remain relatively stable, with changes in urinary profile predominantly due to increases in DMA and lowering of MMA (Hopenhayn et al., 2003b). Previous research has also demonstrated relatively stable urinary methylation profiles within non-pregnant individuals over time (albeit with limited measurements) (Concha et al., 2002; Tseng, 2009). Additionally, stability in absolute iAs concentrations in urine has been demonstrated with folate supplementation (Gamble et al., 2006).

Here we found that low birth weight outcome was informed solely based on absolute urinary iAs concentration values. Moreover, because our data examined the underlying, background metabolic fingerprint for iAs exposure in non-pregnant women, our preliminary evidence suggests urinary iAs concentration may be an attractive biomarker to predict and convey information regarding fetal health risk. The utility of urinary iAs as a biomarker is supported by previous research demonstrating low variability in mean urinary iAs concentrations between residents of areas with low and high iAs exposure through drinking water but relatively high variability between individuals within the same area (Hopenhayn-Rich et al., 1996b). This suggests that exposure is not the primary factor in determining metabolic partitioning of urinary arsenic metabolites.

To our knowledge, the idea of inter-individual differences in partitioning between arsenic methylation destined for excretion, and retention has not previously been investigated as a modifier of diverse health outcomes, with the exception of skin lesions (Ghosh et al., 2007). In our study, the exposed women had similar long-term moderate levels of iAs exposure through drinking water. The underlying metabolic trafficking pattern revealed greater excretion in the low birth weight outcome group, suggesting the possibility that competition for limited methyl units during pregnancy may compromise fetal growth. In contrast,

because toenail arsenic concentrations did not prove distinct between the exposed women, we interpret these findings to suggest that those women with normal birth weight children likely partition more iAs toward a central compartment with slow turnover, *e.g.*, liver, perhaps tempering the use of methyl units. Recent work modeling iAs metabolic pathways demonstrates the necessity of a slow, reversible liver pool to explain the human exposure data (Lawley et al., 2011). This underlying metabolic fingerprint may help explain the relative stability in intra-individual urine iAs under conditions where overall iAs exposures are constant, but inter-individual variability is high.

Our findings help to shed some light on the discord among studies of iAs exposure and birth weight. It would seem that not only differences in exposure magnitude, but also differences in iAs metabolism, specifically high partitioning toward excretion, may influence birth weight outcomes. We acknowledge that it is possible that differences in our study may be due to nutritional status differences of the women studied. However, we believe that is unlikely to account for the findings as the women in this study are all of healthy BMI, using criteria defined by a recent study of nutritional status in a Romanian population (Nedo and Paulik, 2012). Moreover, the majority of women reported pre-natal vitamin use, indicating awareness of good nutritional status for a healthy pregnancy, and all women were under medical care during their pregnancy. Additionally, voluntary fortification of the food supply is widely practiced in the EU, including Romania, under the regulation 1925/2006/EC which includes the fortification of all foods excluding alcohol and unrefined foods (EFSA, 2009).

Potential confounders such as smoking (Chen et al., 2005; Hertz-Picciotto et al., 1992), occupational exposure to arsenic (Chen et al., 2005; Hertz-Picciotto et al., 1992), and number of prior pregnancies did not vary between the two group, and while education varied, iAs exposure did not. Dietary sources of iAs in the region of Romania studied are expected to be insignificant based on the food frequency questionnaire in the ASHRAM study (Hough et al., 2010). No other significant sources of arsenic, such as from industrial or mining activities, were identified in the region.

There are no existing data that consider a woman's underlying iAs metabolism with respect to reproductive outcome. Our metabolic study used comprehensive individual measurements of arsenic concentrations in drinking water sources, urine, and toenails to maximize precision in exposure assessment and metabolism. Our pilot, metabolic sample was similar in sample size to other studies (Hopenhayn et al., 2003b). We acknowledge the possibility for attenuation bias (Sobus et al., 2010) in that it is possible that one biomarker measurement may not truly reflect variance over time within the individual; however most studies of iAs methylation do use a single urine and toenail sample to estimate exposure and to investigate various health outcomes (Ahsan et al., 2007; Heck et al., 2009; Steinmaus et al., 2010). Moreover, our study takes advantage of long-standing residence status and stable Romanian ground water arsenic levels; taken together this stability supports long-standing metabolic equilibrium of iAs in both the primary tissues and body fluids (Adeyemi et al., 2010).

5. Conclusion

Our findings provide evidence that inter-individual differences in underlying iAs metabolic partitioning at low-to-moderate levels of exposure may contribute to altering birth weight outcomes in women. Further prospective research before and during pregnancy is needed to refine the estimate, but preliminary evidence based on absolute urine iAs concentrations suggest that values greater than 9.0 μg/L may provide a cautionary limit for women consuming low-to-moderate amounts of iAs through drinking water. Low-to-moderate levels of iAs exposure through drinking water are common globally. Determining the underlying metabolic partitioning pattern of iAs may help to identify vulnerable populations

and inform interventions to lessen the health burden of low birth weight outcome in iAs endemic regions.

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Abbreviations

ANOVA	analysis of variance
ASHRAM	Arsenic Health Risk Assessment and Molecular Epidemiology
DMA	dimethylarsinic acid
EU	European Union
iAs	inorganic arsenic
MMA	monomethylarsonic acid

References

Adeyemi A, Garelick H, Priest ND. A biokinetic model to describe the distribution and excretion of arsenic by man following acute and chronic intakes of arsenite/arsenate compounds by ingestion. Human & experimental toxicology. 2010; 29:891–902. [PubMed: 20219843]

Ahmad SA, Sayed MH, Barua S, Khan MH, Faruquee MH, Jalil A, Hadi SA, Talukder HK. Arsenic in drinking water and pregnancy outcomes. Environ Health Perspect. 2001; 109:629–631. [PubMed: 11445518]

Ahsan H, Chen Y, Kibriya MG, Slavkovich V, Parvez F, Jasmine F, Gamble MV, Graziano JH. Arsenic metabolism, genetic susceptibility, and risk of premalignant skin lesions in Bangladesh. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2007; 16:1270–1278.

Chen YC, Guo YL, Su HJ, Hsueh YM, Smith TJ, Ryan LM, Lee MS, Chao SC, Lee JY, Christiani DC. Arsenic methylation and skin cancer risk in southwestern Taiwan. J Occup Environ Med. 2003a; 45:241–248. [PubMed: 12661181]

Chen YC, Su HJ, Guo YL, Houseman EA, Christiani DC. Interaction between environmental tobacco smoke and arsenic methylation ability on the risk of bladder cancer. Cancer Causes Control. 2005; 16:75–81. [PubMed: 15868449]

Chen YC, Su HJ, Guo YL, Hsueh YM, Smith TJ, Ryan LM, Lee MS, Christiani DC. Arsenic methylation and bladder cancer risk in Taiwan. Cancer Causes Control. 2003b; 14:303–310. [PubMed: 12846360]

Concha G, Vogler G, Lezcano D, Nermell B, Vahter M. Exposure to inorganic arsenic metabolites during early human development. Toxicol Sci. 1998; 44:185–190. [PubMed: 9742656]

Concha G, Vogler G, Nermell B, Vahter M. Intra-individual variation in the metabolism of inorganic arsenic. Int Arch Occup Environ Health. 2002; 75:576–580. [PubMed: 12373320]

Del Razo LM, Garcia-Vargas GG, Vargas H, Albores A, Gonsebatt ME, Montero R, Ostrosky-Wegman P, Kelsh M, Cebrian ME. Altered profile of urinary arsenic metabolites in adults with chronic arsenicism. A pilot study. Arch Toxicol. 1997; 71:211–217.

EFSA. [10 March 2012] ESCO report prepared by the EFSA Scientific Cooperation Working Group on Analysis of Risks and Benefits of Fortification of Food with Folic Acid.. European Food Safety Authority. 2009. Available at www.efsa.europa.eu/en/scdocs/doc/3e.pdf.

El-Masri HA, Kenyon EM. Development of a human physiologically based pharmacokinetic (PBPK) model for inorganic arsenic and its mono- and di-methylated metabolites. Journal of pharmacokinetics and pharmacodynamics. 2008; 35:31–68. [PubMed: 17943421]

Gamble MV, Liu X, Ahsan H, Pilsner JR, Ilievski V, Slavkovich V, Parvez F, Chen Y, Levy D, Factor-Litvak P, Graziano JH. Folate and arsenic metabolism: a double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. The American journal of clinical nutrition. 2006; 84:1093–1101. [PubMed: 17093162]

Gardner RM, Nermell B, Kippler M, Grander M, Li L, Ekstrom EC, Rahman A, Lonnerdal B, Hoque AM, Vahter M. Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status. Reprod Toxicol. 2011; 31:210–218. [PubMed: 21078382]

Ghosh P, Banerjee M, De Chaudhuri S, Chowdhury R, Das JK, Mukherjee A, Sarkar AK, Mondal L, Baidya K, Sau TJ, Banerjee A, Basu A, Chaudhuri K, Ray K, Giri AK. Comparison of health effects between individuals with and without skin lesions in the population exposed to arsenic through drinking water in West Bengal, India. Journal of exposure science & environmental epidemiology. 2007; 17:215–223. [PubMed: 16835595]

Gurzau, ES.; Gurzau, AE. Arsenic in drinking water from groundwater in Transylvania, Romania: An overview. In: Chappell, WR.; Abernathy, CO.; Calderon, RL., editors. Arsenic Exposure and Health Effects IV. New York. Elsevier Science; 2001. p. 181-184.

Hall M, Gamble M, Slavkovich V, Liu X, Levy D, Cheng Z, van Geen A, Yunus M, Rahman M, Pilsner JR, Graziano J. Determinants of arsenic metabolism: blood arsenic metabolites, plasma folate, cobalamin, and homocysteine concentrations in maternal-newborn pairs. Environ Health Perspect. 2007; 115:1503–1509. [PubMed: 17938743]

Heck JE, Andrew AS, Onega T, Rigas JR, Jackson BP, Karagas MR, Duell EJ. Lung cancer in a U.S. population with low to moderate arsenic exposure. Environ Health Perspect. 2009; 117:1718–1723. [PubMed: 20049123]

Hertz-Picciotto I, Smith AH, Holtzman D, Lipsett M, Alexeeff G. Synergism between occupational arsenic exposure and smoking in the induction of lung cancer. Epidemiology. 1992; 3:23–31. [PubMed: 1554806]

HFA-DB. European Health for All Database. World Health Organization, Regional Office for Europe; 2012. Available at http://www.euro.who.int/hfadb. [10 March 2012]

Hopenhayn-Rich C, Biggs ML, Kalman DA, Moore LE, Smith AH. Arsenic methylation patterns before and after changing from high to lower concentrations of arsenic in drinking water. Environ Health Perspect. 1996a; 104:1200–1207. [PubMed: 8959409]

Hopenhayn-Rich C, Biggs ML, Smith AH, Kalman DA, Moore LE. Methylation study of a population environmentally exposed to arsenic in drinking water. Environ Health Perspect. 1996b; 104:620–628. [PubMed: 8793350]

Hopenhayn C, Ferreccio C, Browning SR, Huang B, Peralta C, Gibb H, Hertz-Picciotto I. Arsenic exposure from drinking water and birth weight. Epidemiology. 2003a; 14:593–602. [PubMed: 14501275]

Hopenhayn C, Huang B, Christian J, Peralta C, Ferreccio C, Atallah R, Kalman D. Profile of urinary arsenic metabolites during pregnancy. Environ Health Perspect. 2003b; 111:1888–1891. [PubMed: 14644662]

Hough RL, Fletcher T, Leonardi GS, Goessler W, Gnagnarella P, Clemens F, Gurzau E, Koppova K, Rudnai P, Kumar R, Vahter M. Lifetime exposure to arsenic in residential drinking water in Central Europe. Int Arch Occup Environ Health. 2010; 83:471–481. [PubMed: 20401490]

Huyck KL, Kile ML, Mahiuddin G, Quamruzzaman Q, Rahman M, Breton CV, Dobson CB, Frelich J, Hoffman E, Yousuf J, Afroz S, Islam S, Christiani DC. Maternal arsenic exposure associated with

low birth weight in Bangladesh. J Occup Environ Med. 2007; 49:1097–1104. [PubMed: 18000415]

INS. Institutul National de Statistica. Bucharest, Romania: 2012a. Arad County Statistics.. Available at http://www.arad.insse.ro. [2 July 2013]

INS. Statistical Yearbook 2011, Chapter 2: Population. Institutul National de Statistica; Bucharest, Romania: 2012b. Available at http://www.insse.ro/cms/rw/pages/anuarstatistic2011.ro.do. [2 July 2013]

Karagas MR, Tosteson TD, Blum J, Klaue B, Weiss JE, Stannard V, Spate V, Morris JS. Measurement of low levels of arsenic exposure: a comparison of water and toenail concentrations. Am J Epidemiol. 2000; 152:84–90. [PubMed: 10901333]

Kenyon EM, Hughes MF, Adair BM, Highfill JH, Crecelius EA, Clewell HJ, Yager JW. Tissue distribution and urinary excretion of inorganic arsenic and its methylated metabolites in C57BL6 mice following subchronic exposure to arsenate in drinking water. Toxicol Appl Pharmacol. 2008; 232:448–455. [PubMed: 18706920]

Kile ML, Hoffman E, Hsueh YM, Afroz S, Quamruzzaman Q, Rahman M, Mahiuddin G, Ryan L, Christiani DC. Variability in biomarkers of arsenic exposure and metabolism in adults over time. Environ Health Perspect. 2009; 117:455–460. [PubMed: 19337522]

Kile ML, Houseman EA, Breton CV, Quamruzzaman Q, Rahman M, Mahiuddin G, Christiani DC. Association between total ingested arsenic and toenail arsenic concentrations. Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering. 2007; 42:1827–1834.

Kitchin KT, Del Razo LM, Brown JL, Anderson WL, Kenyon EM. An integrated pharmacokinetic and pharmacodynamic study of arsenite action. 1. Heme oxygenase induction in rats. Teratogenesis, carcinogenesis, and mutagenesis. 1999; 19:385–402.

Kurttio P, Komulainen H, Hakala E, Kahelin H, Pekkanen J. Urinary excretion of arsenic species after exposure to arsenic present in drinking water. Arch Environ Contam Toxicol. 1998; 34:297–305. [PubMed: 9504979]

Lawley SD, Cinderella M, Hall MN, Gamble MV, Nijhout HF, Reed MC. Mathematical model insights into arsenic detoxification. Theoretical biology & medical modelling. 2011; 8:31. [PubMed: 21871107]

Leonardi G, Vahter M, Clemens F, Goessler W, Gurzau E, Hemminki K, Hough R, Koppova K, Kumar R, Rudnai P, Surdu S, Fletcher T. Inorganic arsenic and basal cell carcinoma in areas of Hungary, Romania, and Slovakia: a case-control study. Environ Health Perspect. 2012; 120:721–726. [PubMed: 22436128]

Lindberg AL, Goessler W, Gurzau E, Koppova K, Rudnai P, Kumar R, Fletcher T, Leonardi G, Slotova K, Gheorghiu E, Vahter M. Arsenic exposure in Hungary, Romania and Slovakia. J Environ Monit. 2006; 8:203–208. [PubMed: 16395480]

Marchiset-Ferlay N, Savanovitch C, Sauvant-Rochat MP. What is the best biomarker to assess arsenic exposure via drinking water? Environment international. 2012; 39:150–171. [PubMed: 22208756]

Nedo E, Paulik E. Association of smoking, physical activity, and dietary habits with socioeconomic variables: a cross-sectional study in adults on both sides of the Hungarian-Romanian border. BMC public health. 2012; 12:60. [PubMed: 22264383]

Rahman A, Vahter M, Ekstrom EC, Rahman M, Golam Mustafa AH, Wahed MA, Yunus M, Persson LA. Association of arsenic exposure during pregnancy with fetal loss and infant death: a cohort study in Bangladesh. Am J Epidemiol. 2007; 165:1389–1396. [PubMed: 17351293]

Scheer J, Findenig S, Goessler W, Francesconi KA, Howard B, Umans JG, Pollak J, Tellez-Plaza M, Silbergeld EK, Guallar E, Navas-Acien A. Arsenic species and selected metals in human urine: validation of HPLC/ICPMS and ICPMS procedures for a long-term population-based epidemiological study. Analytical methods : advancing methods and applications. 2012; 4:406–413. [PubMed: 22685491]

Smith AH, Steinmaus CM. Health effects of arsenic and chromium in drinking water: recent human findings. Annual review of public health. 2009; 30:107–122.

Sobus JR, Pleil JD, McClean MD, Herrick RF, Rappaport SM. Biomarker variance component estimation for exposure surrogate selection and toxicokinetic inference. Toxicology letters. 2010; 199:247–253. [PubMed: 20851754]

Steinmaus C, Yuan Y, Kalman D, Rey OA, Skibola CF, Dauphine D, Basu A, Porter KE, Hubbard A, Bates MN, Smith MT, Smith AH. Individual differences in arsenic metabolism and lung cancer in a case-control study in Cordoba, Argentina. Toxicol Appl Pharmacol. 2010; 247:138–145. [PubMed: 20600216]

Tseng CH. A review on environmental factors regulating arsenic methylation in humans. Toxicol Appl Pharmacol. 2009; 235:338–350. [PubMed: 19168087]

Vahter M. Mechanisms of arsenic biotransformation. Toxicology 181. 2002; 182:211–217.

Vahter M. Effects of arsenic on maternal and fetal health. Annu Rev Nutr. 2009; 29:381–399. [PubMed: 19575603]

Yang CY, Chang CC, Tsai SS, Chuang HY, Ho CK, Wu TN. Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in northeastern Taiwan. Environ Res. 2003; 91:29–34. [PubMed: 12550085]

Highlights

- Women in an inorganic arsenic (iAs) endemic area show distinct metabolic patterns.
- Underlying IAs methylation efficiency did not distinguish birth weight outcome.
- Underlying partitioning of iAs toward excretion characterized birth weight outcome.
- Evidence suggests a potential urinary iAs biomarker for low birth weight outcome.

Institutional Review Board Approval and Policy Adherence

The study protocol was approved by the institutional review boards of the regional public health authority of Arad County in Romania (Centrul Regional de Sănătate Publică Timişoara, registration number 206/26.04.2010) and Yale University School of Medicine Human Investigation Committee (HIC Protocol Number 1004006636). The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and Uniform Requirements for manuscripts submitted to Biomedical journals.

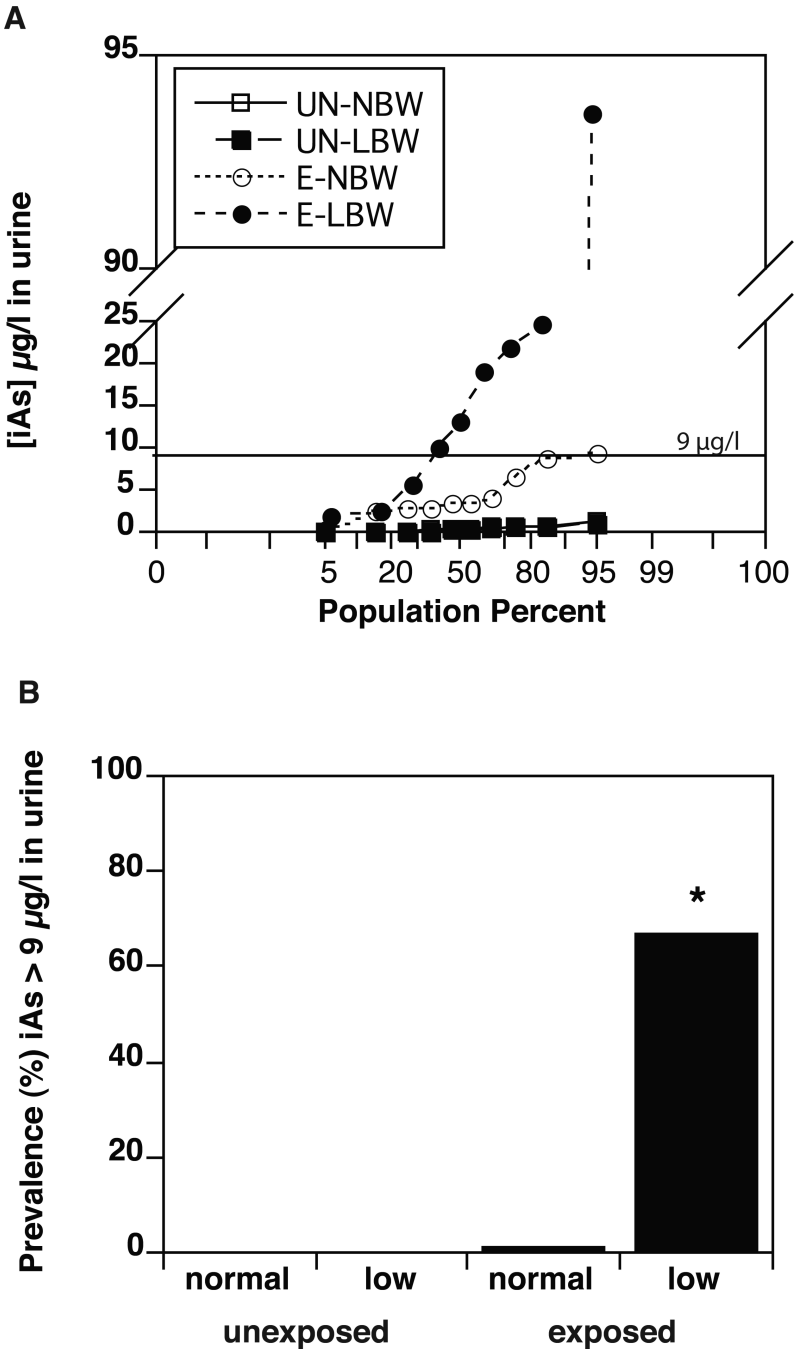

Figure 1.

Multivariate ANOVA showing main effects (*) ($p<0.05$) of exposure and birth weight outcome (normal or low): (A) inorganic arsenic (iAs) drinking water exposure, methylation efficiency, (B) primary methylation index (PMI), (C) secondary methylation index (SMI), and (D) peripheral (toenail) storage. Main effects of exposure were observed for all parameters ($p<0.05$), except for PMI. There were neither significant main effects of birth weight nor significant interaction effects for these variables. Within the exposed and unexposed groups, the total ingested arsenic dose from drinking water showed no significant difference.

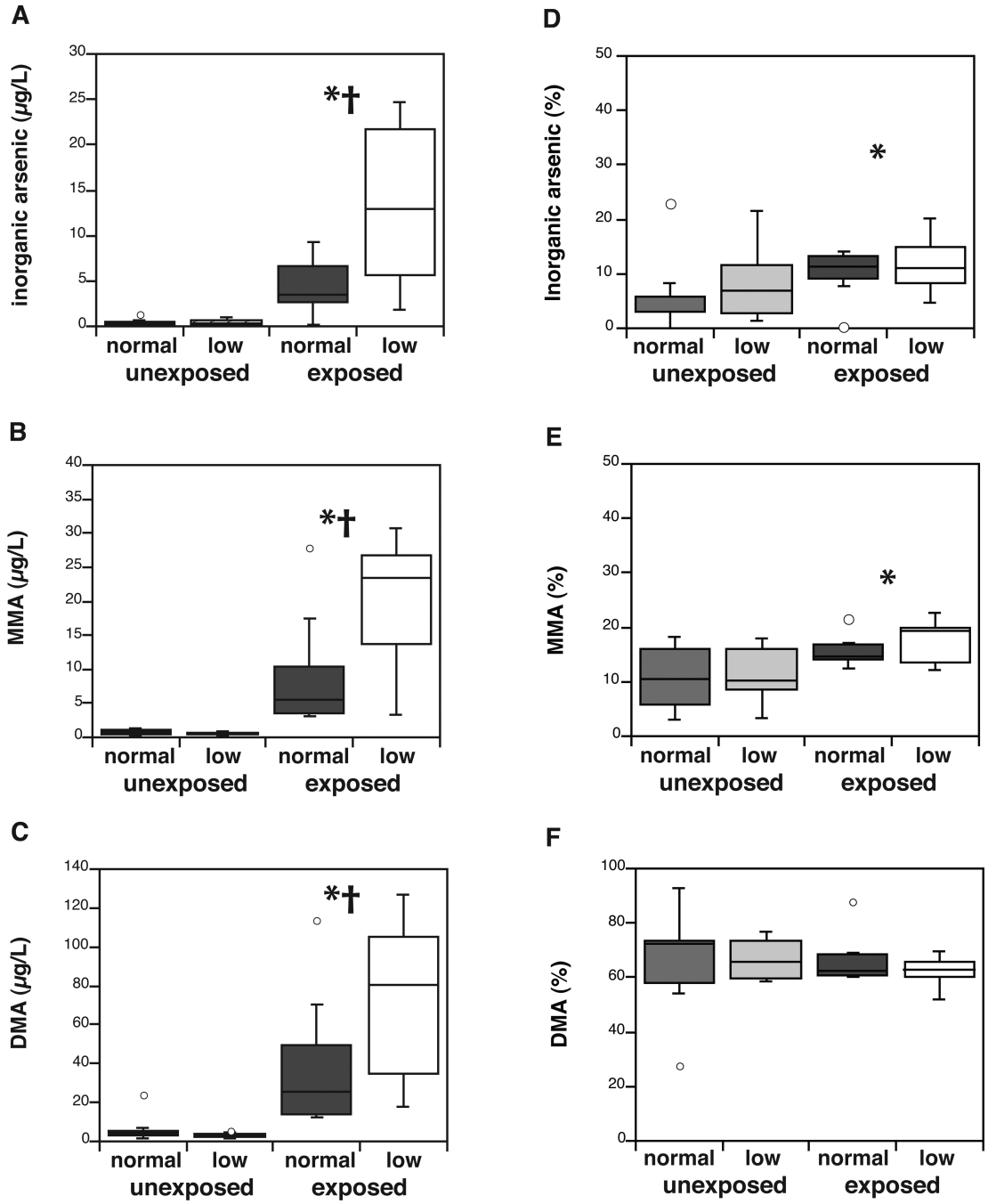

Figure 2.

Multivariate ANOVAs showing main effects (*) and interactive effects (†) ($p<0.05$) of exposure and birth weight outcome (normal or low) with all urine variables in the models: (A) inorganic arsenic, (B) monomethylarsonic acid (MMA), and (C) dimethylarsinic acid (DMA), and (D) % inorganic arsenic, (E) % MMA, and (F) % DMA. This analysis revealed significance of all factors as a main effect of exposure ($p<0.001$). Significant interaction effects between exposure and birth weight were seen for all absolute urine metabolites: DMA ($p=0.015$), MMA ($p=0.018$), and iAs ($p=0.020$) that demonstrated increased excretion for the exposed, low birth weight group. When percentages of the metabolites were used, the

only significant increases were for the main effect of exposure for MMA(%) ($p<0.001$) and iAs(%) ($p=0.043$).

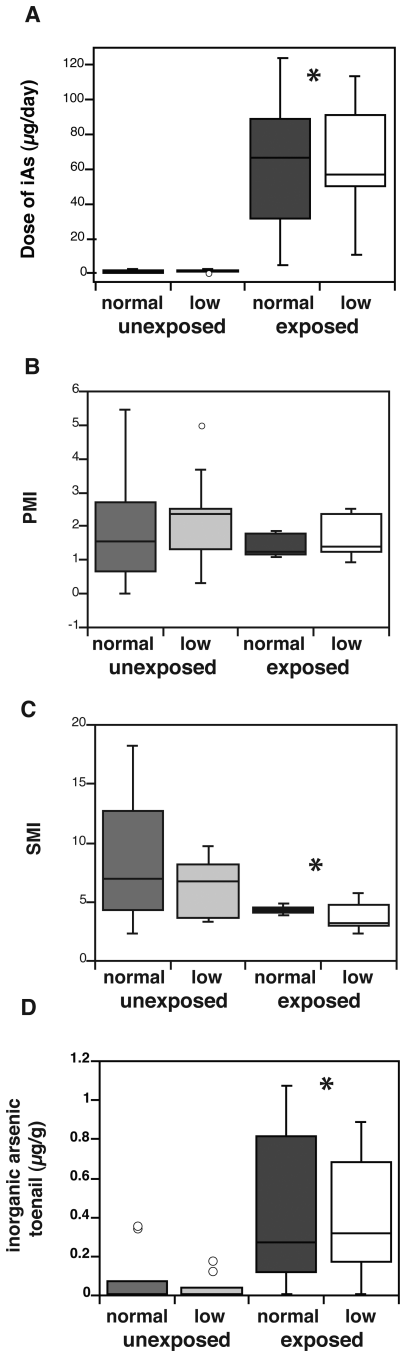

Figure 3.

Graphs showing (A) breakdown of subjects with urinary iAs > 9.0 μg/L by exposure and birth weight outcome (UN: unexposed; E: exposed; NBW: normal birth weight; LBW: low birth weight) and (B) percent of subjects by exposure and birth weight outcome (normal or low) with > 9.0 μg/L urinary iAs. Sixty-seven percent of exposed women in the low birth weight group compared to only 10% of exposed women in the normal birth weight group (p=0.019) had urinary iAs concentrations > 9.0 μg/L.

Table 1

Characteristics of study participants by exposure group.[a]

Unexposed Study Participants			
Characteristic	**NBW[b] (n=10)**	**LBW[c] (n=9)**	**p-value[d]**
Age (years)	30.2 ± 3.0	30.6 ± 3.1	0.80
Birth weight of child (g)	3352.0 ± 352.5	2355.6 ± 104.4	<0.01
BMI (kg/m^2)	22.7 ± 3.8	22.8 ± 3.9	0.94
Education (years)	13.9 ± 2.4	11.9 ± 4.8	0.25
Fasting blood glucose, 3rd trimester (mg/dL)	85.4 ± 17.1	86.0 ± 11.2	0.93
Number of prior pregnancies	1.2 ± 1.0	1.9 ± 2.0	0.35
Use of prenatal vitamins (%)	80.0 (8)	77.8 (7)	1.00
Current smoker (%)	10.0 (1)	33.3 (3)	0.30
Smoked during pregnancy (%)	10.0 (1)	33.3 (3)	0.30
Consumes alcohol (%)	0.0 (0)	0.0 (0)	NA

Exposed Study Participants			
Characteristic	**NBW[b] (n=10)**	**LBW[c] (n=9)**	**p-value[d]**
Age (years)	31.1 ± 5.5	26.6 ± 5.4	0.86
Birth weight of child (g)	3470.0 ± 222.6	2405.6 ± 212.8	<0.01
BMI (kg/m^2)	24.5 ± 3.2	21.9 ± 3.4	0.11
Education (years)	10.6 ± 2.4	6.6 ± 3.8	0.02
Fasting blood glucose, 3rd trimester (mg/dL)	74.0 ± 5.2	76.3 ± 3.4	0.27
Number of prior pregnancies	1.1 ± 1.3	1.6 ± 2.2	0.58
Use of prenatal vitamins (%)	80.0 (8)	66.7 (6)	0.63
Current smoker (%)	20.0 (2)	11.1 (1)	1.00
Smoked during pregnancy (%)	10.0 (1)	11.1 (1)	1.00
Consumes alcohol (%)	0.0 (0)	0.0 (0)	NA

[a] Values are mean ± SD for continuous variables and column % (n) for categorical variables.

[b] NBW = Normal Birth Weight

[c] LBW = Low Birth Weight

[d] P-value is for t-test (continuous variables) or χ^2 test/Fisher's exact test (categorical variables).

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Table 2

Partial correlation coefficients by birth weight outcome.[a]

	Exposure (drinking water conc.)		Dose		Urinary iAs		Urinary DMA		Urinary MMA		Toenail	
	NBW	LBW	NBW	LBW	NBW	LBW	NBW	LBW	NBW	LBW	NBW	LBW
Expos.			**0.989**	**0.959**	**0.627**	**0.866**	**0.753**	**0.788**	**0.771**	**0.701**	**0.724**	**0.689**
Dose	**0.989**	**0.959**			**0.594**	**0.897**	**0.727**	**0.787**	**0.745**	**0.754**	**0.662**	**0.708**
iAs	**0.627**	**0.866**	**0.594**	**0.897**			0.434	**0.910**	0.432	**0.835**	0.395	0.525
DMA	**0.753**	**0.788**	**0.727**	**0.787**	0.434	**0.910**			**0.989**	**0.895**	**0.655**	0.448
MMA	**0.771**	**0.701**	**0.745**	**0.754**	0.432	**0.835**	**0.989**	**0.895**			**0.698**	0.409
Toenail	**0.724**	**0.689**	**0.662**	**0.708**	0.395	0.525	**0.655**	0.448	**0.698**	0.409		

[a]Significance ($p<0.05$) indicated by bolded text. Values are controlled for body mass index, smoking status, and age. iAs = inorganic arsenic, DMA = dimethylarsinic acid, MMA = monomethylarsonic acid, Toenail = toenail iAs concentration, and Dose = iAs exposure concentration*water consumed. NBW = normal birth weight and LBW = low birth weight.