Arsenic Metabolism in Children Differs From That in Adults

Helena Skröder Löveborn,* Maria Kippler,* Ying Lu,* Sultan Ahmed,*,† Doris Kuehnelt,‡ Rubhana Raqib,† and Marie Vahter*,1

*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, SE-171 77, Sweden; †International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka 1000, Bangladesh; and ‡Institute of Chemistry, Analytical Chemistry, NAWI Graz, University of Graz, Universitätsplatz 1, Graz 8010, Austria

1To whom correspondence should be addressed. Fax: +08-336981. E-mail: marie.vahter@ki.se

ABSTRACT

Arsenic toxicity in adults is associated with methylation efficiency, influenced by factors such as gender, genetics, and nutrition. The aim of this study was to evaluate influencing factors for arsenic metabolism in children. For 488 children (9 years), whose mothers participated in a study on arsenic exposure during pregnancy (nested into the MINIMat trial) in rural Bangladesh, we measured urinary concentrations of inorganic arsenic (iAs) and its metabolites methylarsonic acid (MMA) and dimethylarsinic acid (DMA) by HPLC-HG-ICPMS. Methylation efficiency was assessed by relative amounts (%) of the metabolites. We evaluated the impact of factors such as maternal urinary metabolite pattern, arsenic exposure, gender, socioeconomic status, season of sampling, and nutritional factors, including erythrocyte selenium (Ery-Se), and plasma folate and vitamin B12.

Children had higher %DMA and lower %iAs in urine compared to their mothers, unrelated to their lower exposure [median urinary arsenic (U-As) 53 vs 78 μg/l]. Surprisingly, selenium status (Ery-Se) was strongly associated with children's arsenic methylation; an increase in Ery-Se from the 5–95th percentile was associated with: +1.8 percentage points (pp) for %iAs (P = .001), +1.4 pp for %MMA (P = .003), and −3.2 pp for %DMA (P < .001). Despite this, Ery-Se was positively associated with U-As (5–95th percentile: +41 μg/l, P = .026). As expected, plasma folate was inversely associated with %iAs (5–95th percentile: −1.9 pp, P = .001) and positively associated with %DMA (5–95th percentile: +2.2 pp, P = .008). Children methylated arsenic more efficiently than their mothers. Also influencing factors, mainly selenium and folate, differed. This warrants further research.

Key words: arsenic; children; metabolism; methylation; selenium; folate

Chronic exposure to inorganic arsenic (iAs) through drinking water and food increases the risk of cancer and numerous non-cancer effects (IARC, 2012), but the susceptibility varies markedly between individuals and populations (Faita et al., 2013). Recent findings indicate that early-life is a critical period for arsenic exposure, giving rise to highly elevated health risks later in life, including cancer and overall morbidity and mortality (Smith et al., 2012, 2013).

A well-established susceptibility factor for arsenic-related adverse health effects in adults is inefficient arsenic metabolism. Arsenic is methylated in the body via the 1-carbon metabolism, and the main metabolites excreted in the urine are methylarsonic acid (MMA) and dimethylarsinic acid (DMA), besides some remaining unmethylated inorganic arsenic. Higher fractions of iAs and MMA in the urine have been associated with higher retention of arsenic (Vahter, 2002) and higher risk of adverse health effects (Chen et al., 2005; Lindberg et al., 2008b; Tseng, 2009). Multiple factors have been shown to influence the metabolism of arsenic in adults, including polymorphisms in the arsenic (+III oxidation state) methyltransferase (AS3MT)
gene (Antonelli et al., 2014; Engstrom et al., 2011), gender (Lindberg et al., 2008a), nutrition (Howe et al., 2014; Li et al., 2008), smoking (Lindberg et al., 2010), and pregnancy (Gardner et al., 2012). In addition, selenium has been associated with the methylation of arsenic in adults (George et al., 2013; Huang et al., 2008; Pilsner et al., 2011), but the results are inconsistent.

Although several recent studies indicate severe health effects of elevated arsenic exposure in children, mainly in the form of impaired immune function (Ahmed et al., 2014; Soto-Pena et al., 2006), growth (Gardner et al., 2013), and cognitive function (Hamadani et al., 2011; Wasserman et al., 2007), little is known about potential susceptibility factors, including the efficiency of arsenic metabolism. In a couple of studies, factors such as plasma homocysteine levels and gender appeared to influence the urinary arsenic metabolite fractions differently in young children (1.5–6.5 years) compared to adults (Fangstrom et al., 2009; Hall et al., 2009). The aim of the present study was to further clarify children’s metabolism of arsenic by assessing influencing factors such as past and present arsenic exposure, gender, socioeconomic status (SES), season of sampling, and nutritional factors including selenium, folic acid, and vitamin B12, in 9-year-old children.

METHODS
Study design
This prospective cohort study is part of our ongoing research concerning effects of arsenic on pregnancy outcomes and child health and development (Vahter et al., 2006), which was nested in a randomized population-based food and micronutrient supplementation trial in pregnancy (MINIMat) in Matlab, a rural area 53 km southeast of Dhaka in Bangladesh (Persson et al., 2012). Between November 2001 and October 2003, the trial enrolled a total of 4436 pregnant women with a wide variation of exposure from all sources, ie, both drinking water and food. Persistent elevated exposure to arsenic.

In a subcohort of 640 children, born from June 2003 to June 2004, we have prospectively evaluated the effects of arsenic exposure on the children’s immune function (Ahmed et al., 2012). Because we barely found any impact of arsenic metabolism on various outcomes (unpublished data; Ahmed et al., 2013; Gardner et al., 2013; Hamadani et al., 2011), we now evaluate factors influencing the metabolism of arsenic in the present study. Details on the selection of these children and the follow-ups have been described in detail previously (Ahmed et al., 2012, 2014). In total, 488 children had both prenatal and concurrent (at 9 years of age) exposure data. We have exposure data for the children also at 4.5 years, but we restricted this study to the follow-up at 9 years as 34% of the children had no blood analyzed at 4.5 years. Importantly, the methylation patterns were very similar at 4.5 and 9 years, and there were no major differences in other characteristics either (Supplementary Table S1).

The study was approved by the Ethical Committees at icddr,b, Bangladesh, and Karolinska Institutet, Sweden. Informed consent was obtained from all the guardians. Participants were free to refrain partially or totally from participation at any time of the study.

Arsenic exposure and metabolism
We assessed individual arsenic exposure based on the sum concentration of iAs and its methylated metabolites (MMA and DMA) in urine, hereinafter referred to as U-As, which reflects exposure from all sources, ie, both drinking water and food. Arsenic methylation efficiency was evaluated based on the relative concentrations (%) of the different arsenic metabolites in urine (Vahter, 2002).

Spot urine samples were collected in early and late pregnancy [gestational week (GW) 8 and 30] as previously described (Vahter et al., 2006). For the purpose of the present study, we have used the urine data from early pregnancy. These provide a more representative pattern of the women’s usual urinary arsenic metabolites, since the methylation of arsenic changes markedly during pregnancy (Gardner et al., 2012; Li et al., 2008). At the follow-up of the children we collected spot urine samples at the health care facilities using plastic urine collection cups tested to be free from trace elements. Urine was immediately transferred to 24 ml polyethylene bottles, which were kept in a refrigerator until they, at the end of the day, were sent to the Matlab hospital laboratory for storage at ~20 °C. Every week, urine samples were transported to the Nutritional Biochemistry laboratory in Dhaka, where they were stored at ~80 °C. At the end of the sampling period, urine samples were transported in cooling boxes on ice to Karolinska Institutet in Stockholm, Sweden, for analysis of arsenic metabolites and other elements.

The concentrations of arsenic metabolites [iAs (III), iAs (V), MMA (V), and DMA (V)] in maternal and child urine were measured using high-performance liquid chromatography on-line coupling with hydride generation inductively coupled plasma mass spectrometry (HPLC-ICP-MS), as described previously (Fangstrom et al., 2008; Gardner et al., 2011a). In short, the urine samples (0.5 ml) were filtered on a 0.2 µm hydrophilic syringe filter (Sartstedt, Germany) and 10 µl was injected on the ion exchange column (Hamilton PXP-X100, 10 µm, 250 × 4.6 mm, Reno, Nevada) of the HPLC system (Agilent 1100 series; Agilent Technologies, Waldbronn, Germany). The limit of detection (LOD) was 0.2 µg/l for inorganic As (III), MMA and DMA, and 0.5 µg/l for inorganic As (V). Nine samples were below LOD for AsIII, 5 for MMA, 21 for AsV, but none for DMA. We used the measured concentrations for these samples, and replacing them with LOD/2 made no difference to the results. The intra-and inter-assay coefficients of variation were both approximately 4%, based on repeated measurements of the quality control material NIES CRM No.18 human urine (National Institute for Environmental Studies, Tsukuba City, Japan; n = 49) during 8 non-consecutive days. The reference value of DMA was 36–9 µg/l (mean ± SD) and our measured mean DMA concentrations were 42–43 (± 1.2–1.6 µg/l), which was in agreement with values obtained previously (Ahmed et al., 2014; Gardner et al., 2011a).

Total arsenic in urine (U-As tot) was measured using ICP-MS (Agilent 7700x, Agilent Technologies, Tokyo, Japan), with the collision/reaction cell in helium mode to minimize interferences (Lu et al., 2015). Before the measurements, the urine samples were diluted 1:10 with 1% nitric acid (65% w/w, Scharlau, Scharlau S.L., Sentmenat, Spain). The LOD was <0.042 µg/l (Supplementary Table S2 and S3), and no samples were below this concentration. The correlation between children’s U-As tot and U-As (sum of iAs metabolites), measured with the 2 methods (HPLC-HG-ICPMS and ICP-MS, respectively) was r As = 0.98 (P < .001), providing additional support for good analytical quality. However, some differences between U-As sum and U-As were found, why we additionally measured arsenobetaine in urine of a subsample of children from the same study population (n = 20) using HPLC-ICPMS, at the University of Graz, Austria. Urine was filtered through 0.2 µm Nylon filters (Markus Bruckner Analysentechnik, Linz, Austria) and 10 µl were
injected onto a cation-exchange column (Ionosphere SC, 100 × 3 mm, Agilent Technologies) placed in an Agilent 1100 series HPLC system. The mobile phase was 10 mM pyridine pH 2.8 (adjusted with formic acid), the flow rate was 1 ml/min, and the column temperature was 30 °C. The outlet of the column was connected to the nebulizer of an Agilent 7500ce ICP-MS. To enhance the arsenic signal, 1% CO2 was added to the argon gas. The LOD for arsenobetaine was 0.5–3 µg As/l, depending on the intensity of the DMA peak eluting before the arsenobetaine.

To compensate for the variation in dilution of the sampled urine, which has a considerable effect on the measured urinary concentrations, we adjusted the obtained concentrations for specific gravity, measured by a digital refractometer (RD 712 Clinical Refractometer, EUROMEX microscopes, Holland). We previously found that adjustment by specific gravity in this population was less affected by age, body size, SES and arsenic exposure, than the more commonly used creatinine adjustment (Nermell et al., 2008). Also, the urinary DMA was found to correlate significantly with urinary creatinine excretion, both being influenced by the 1-carbon metabolism. All urinary concentrations were adjusted to the overall mean specific gravity, which was 1.012 for both maternal and child urine (range 1.002–1.031). In a subsample of the studied mothers (n = 30), we also measured the osmolality by a digital cryoscopic osmometer (OSMOMAT 030, Gonotec Gesellschaft für Meß- und Regeltechnik mbH, Berlin, Germany) and found a strong correlation with the specific gravity at GW8 (r = 0.98, P < .001), supporting appropriate adjustment for the variation in dilution.

Potential factors influencing arsenic metabolism

Maternal anthropometry, parity, education and SES of the families were collected in early pregnancy at enrollment in the MINIMat trial (Persson et al., 2012). Socioeconomic status was estimated via an asset index, generated through principal component analysis of household assets (on the basis of household ownership of different items) including land, dwelling characteristics, and household sanitation. At the follow-up at 9 years of age, the children’s body weight was measured to the nearest 0.1 kg with a digital scale (TANITA HD - 318, Tanita Corporation, Japan), and height was measured with a locally manufactured wooden stadiometer (precise to 0.1 cm). The measured height and weight were converted to height-for-age (HAZ), weight-for-age (WAZ), and BMI-for-age z-scores (BAZ; SD scores), using the WHO growth reference for school-aged children and adolescents (http://www.who.int/growthref/tools/en/). Stunting was defined as HAZ < −2 (below minus 2 standard deviations from median HAZ of the reference population), and underweight was defined as WAZ < −2 (below minus 2 standard deviations from median WAZ of the reference population). Season of urine and blood sampling was defined as pre-monsoon (January–May), monsoon (June–September), or post-monsoon (October–December). As additional markers of the nutritional status of the children, we measured selenium, zinc, copper, and manganese in blood (erythrocyte fraction) and folate, and vitamin B12 in plasma. Venous blood was collected in Na-heparin treated trace element free tubes (Vacutette, Greiner Bio-One International AG, Kremsmünster, Austria) at the health care facilities and transported to the Matlab hospital laboratory for separation of plasma and erythrocytes.

Concentrations of the elements in erythrocytes were measured using ICP-MS (Agilent 7700x, Agilent Technologies, Tokyo, Japan) after dilution of the erythrocytes in a 1:25 alkali solution consisting of 1-butanol 2% (w/v; anhydrous, 99.8%, Sigma-Aldrich, Schnelldorf, Germany), EDTA 0.05% (w/v; H4-EDTA, 99.995%, Sigma-Aldrich, Schnelldorf, Germany), triton X-100 0.05% (w/v; BioXtra, Sigma-Aldrich, Schnelldorf, Germany), and NH4OH 1% (w/v; Merck, Darmstadt, Germany) (Lu et al., 2015). For comparison, we also measured selenium in urine (U-Se) using ICP-MS (as described above for U-Astot but with the collision/reaction cell in hydrogen mode), and erythrocyte arsenic (Ery-As). For selenium, the LOD in both erythrocytes and urine was below 0.006 µg/l (Supplementary Tables S2 and S3). Obtained values for selenium in reference samples were in good agreement with reference values (Supplementary Tables S2 and S3). Preferably, selenium measured in blood should be expressed per g hemoglobin (Hb) (Kröder et al., 2015; Stefanowicz et al., 2013) and was done so in the present study. Still, we found a strong correlation between Hb-adjusted and unadjusted erythrocyte selenium (Ery-Se) in the children (r = 0.83, P < .001; n = 488).

Folate and vitamin B12 was measured in plasma by electrochemiluminescence immunoassay (ECLIA) using an automated immunoassay analyzer (Cobas e601, Roche Diagnostics, Mannheim, Germany). Quality control serum samples (PreciControl Varia 1 and 2, Roche Diagnostics, Mannheim, Germany) were analyzed in each run to check accuracy and precision. The relative coefficient of variation was <5%.

Statistical analyses

Statistical analyses were conducted using the software Stata/IC, version 12.1 (StataCorp, Texas). Data distribution was evaluated by scatter plots and histograms (Figure 1). Associations between the different arsenic metabolites (iAs, MMA, and DMA) and covariates (mother’s age, education, BMI, and family SES, children’s height, weight, HAZ, WAZ, and BAZ, gender, season of sampling, U-As, Hb, erythrocyte zinc, copper, manganese, Ery-Se, U-Se, and plasma vitamin B12, and folate) were initially evaluated using Spearman’s rank correlation coefficients (for continuous variables) and Mann–Whitney U test or Kruskal–Wallis test (for categorical variables).

We further assessed the associations between the metabolites (as well as U-As and Ery-As) and the covariates using multi-variable-adjusted linear regression analyses. The limit for collinearity was set at t = 0.60, and all regression models were checked with normality and residual versus fitted plots. Covariates included in the final model were factors reported to affect arsenic methylation that were also significantly associated with any of the metabolites in the bivariate analysis, creating the best fitted model for the majority of the outcomes (ie forward selection and backward elimination for each metabolite). These covariates were maternal education, age, SES and % of the assessed metabolite, children’s age, HAZ, season of sampling, Ery-Se, U-Se, and plasma folate. Children’s HAZ was added as stunted/normal height, since there appeared to be an association between HAZ and MMA only in stunted children (Supplementary Figure S1). Maternal % of the assessed metabolite in early pregnancy was added to assess the extent of heritability of arsenic methylation capacity. Concentrations of U-As were added to the model with a spline knot at 58 g/l, below which the arsenic mainly originates from food (mainly rice) (Lindberg et al., 2008a; Vahter et al., 2006), known to be partly in the form of DMA (Meharg et al., 2008). Thus, the metabolism of iAs can only be properly evaluated at exposure levels >58 g/l in this population. The value for the U-As spline knot was set at 58 g/l as this was implied both by the turning point of the scatter plot for %DMA and U-As (Figure 2) and by the intercept for the association between U-As and water arsenic at 9 years (n = 221;
data not shown). We did not include other elements measured in erythrocytes (zinc, copper, manganese), or Hb, in the models as they had no impact on the arsenic methylation (data not shown). To assess potential differences in influential factors depending on the source of arsenic, we also stratified the analyses on U-As below and above 58 μg/l. We also assessed the influence of all factors on the total arsenic concentrations, using U-As and Ery-As as the outcomes instead of the metabolites.

In sensitivity analyses, we additionally adjusted for prenatal U-As and urinary selenium (U-Se at GW8; Ery-Se in pregnancy was not used as this was only available for 136 mothers). Also, we tried adjusting for Ery-As instead of U-As. Since the percentages of the metabolites follow a beta distribution, we also applied Dirichlet multivariate regression. This is an extension of the beta regression which includes all proportions (in this case the %iAs, MMA, and DMA) in the same model.

Based on experimental studies, it has been suggested that selenium and arsenic could form a complex (Berry and Galle, 1994; Levander, 1977), excreted via bile or urine. To assess the potential existence of such a complex, we tested whether the ratio of total urinary arsenic (U-As\textsubscript{tot}; measured with ICP-MS) over the sum of metabolites of iAs (U-As; measured with HPLC-ICPMS) was associated with the concentration of urinary selenium.

RESULTS

The basic characteristics and arsenic metabolite pattern of the studied children at 9 years and their mothers during pregnancy are described in Table 1. There was no difference in the U-As concentration or the relative amounts of the metabolites between boys and girls. Arsenic in children’s erythrocytes and urine were strongly correlated ($r_s = 0.79$, $P < .001$). Although the children’s concentrations of U-As were positively correlated with those of the mothers in early pregnancy (Table 2), the median U-As concentration for the children (53 μg/l, range 9–1268 μg/l) was lower than their mothers’ concentration (77 μg/l, range 2–2063 μg/l; $P < .001$). The children also had significantly lower urinary %iAs and higher %DMA compared to their mothers at
children were much weaker (Table 2). In fact, the children in the highest quartile of U-As (median 192 μg/l) had significantly higher %DMA (82%) compared with the mothers in the lowest quartile of U-As (median 27 μg/l; 78% DMA, P < .001).

Both the children’s plasma folate and U-Se concentrations were inversely correlated with their urinary %iAs and positively associated with %DMA, but not associated with %MMA (Table 2). In contrast, Ery-Se was positively associated with both %iAs and %MMA, and inversely associated with %DMA (Figure 3). The concentrations of Ery-Se and U-Se were poorly correlated (r = 0.090, P = .046). Other essential elements measured in erythrocytes (manganese, zinc, copper), plasma vitamin B12, and Hb, did not correlate with any of the metabolites and were thus not included in the following analyses.

In the multivariable-adjusted linear regression models with a spline knot for U-As at 58 μg/l, the children’s urinary %iAs was found to be higher at the monsoon season than at pre-monsoon, and positively associated with their Ery-Se and maternal %iAs (Table 3). An increase in Ery-Se from the 5th to the 95th percentile (0.38–0.61 μg/g Hb) increased %iAs by almost 2 percentage points (pp), corresponding to about 0.5 SD. On the contrary, the associations with U-As (below spline knot at 58 μg/l), U-Se (μg/l), and folate (μg/l) were inverse. An increase in U-As and plasma folate from the 5th to the 95th percentile (7.9–27 μg/l for U-As and 6.4–15 μg/l for plasma folate) was associated with a ~2 pp decrease in %iAs (P < .001 for both). When stratifying by low/high U-As (below or above 58 μg/l), it appeared that the season of sampling (monsoon: +3.3 pp; P < .001, compared to pre-monsoon) and U-As (−0.66 pp per 10 μg/l; P = .001) had a considerably stronger influence on %iAs below 58 μg/l (mainly reflecting exposure to iAs and DMA though food) than above (Supplementary Table S4). The main influencing factor above 58 μg/l (mainly reflecting exposure to iAs through drinking water) was Ery-Se (+2.5 pp from the 5th to the 95th percentile; P < .001). U-As (range 58–1268 μg/l in the high stratum) was not significantly associated with %iAs (Supplementary Table S4).

The %MMA was positively associated with Ery-Se (Figure 3), SES, maternal education, and child U-As (above spline knot at 58 μg/l; Table 3). Again, selenium was strongly influential; an increase in Ery-Se from the 5th to the 95th percentile increased %MMA by 1.4 pp (−0.4 SD). The increase in %MMA for the same increase in SES (5th to 95th percentile) also corresponded to about 0.4 SD. For U-As (above the spline knot at 58 μg/l; Table 2).

GW8 (P < .001; Figure 1), and the differences were less obvious at the lower exposure levels (U-As < 58μg/l with exposure mainly through food). Also, the mothers’ U-As was inversely correlated with their urinary %DMA and positively correlated with their %iAs and %MMA. The corresponding correlations in the

**Table 1** Basic Characteristics and Urinary Arsenic Metabolites of the Studied Children and Mothers (n = 488)

| Characteristic | GW8a | 9 yearsa |
|---------------|------|---------|
| Age (years)   | 26 ± 5.9 | 8.9 ± 0.11 |
| Height (cm)   | 150 ± 53 | 124 ± 5.4 |
| Weight (kg)   | 46 ± 7.7 | 22 ± 5.6 |
| BMI (kg/m²)   | 21 ± 2.9 | 14 ± 1.7 |
| SES           | 0.88 (3.6, 3.0) | −0.15 (−1.4, 2.0) |
| Parity        | 1.4 ± 1.3 | 0.10 ± 0.17 |
| Sex (%male/female) | 0/100 | 49/51 |
| HAZ (z-score) | −1.4 ± 0.89 | |
| WAZ (z-score) | −1.7 ± 1.1 | |
| BAZ (z-score) | −1.3 ± 1.1 | |
| U-As (μg/l)b | 78 (20–679) | 53 (19–361) |
| Ery-As (μg/kg) | 4.8 (1.5–20)c | 3.3 (1.3–19) |
| U-Se (μg/l)b | 9.4 (5.0–19) | 14 (7.9–27) |
| Ery-Se (μg/kg) | 158 (117–218)c | 172 (139–213) |
| Ery-Zn (μg/kg) | 0.47 (0.35–0.69)d | 0.48 (0.38–0.61) |
| Ery-Cu (μg/kg) | 9035 (5602–10 994)e | 9344 (6856–11 618) |
| Ery-Mn (μg/kg) | 942 (718–1319)f | 646 (560–754) |
| Folate (μg/l) | — | 23 (15–37) |
| Vitamin B12 (μg/l) | — | 639 ± 241i |
| %iAs | 14 (6.2–30) | 8.2 (4.5–17) |
| %MMA | 9.5 (4.7–17) | 8.9 (4.6–16) |
| %DMA | 76 (56–87) | 82 (72–90) |

**Abbreviations:** BAZ, BMI-for-age z-score; BMI, body mass index; DMA, dimethylarsinic acid; Ery-As, erythrocyte arsenic; Ery-Cu, erythrocyte copper; Ery-Mn, erythrocyte manganese; Ery-Se, erythrocyte selenium; Ery-Zn, erythrocyte zinc; HAZ, height-for-age z-score; iAs, inorganic arsenic; MMA, methylarsonic acid; SES, Socioeconomic status; U-As, Urinary arsenic; WAZ, weight-for-age z-score.

Values are shown as mean ± SD, median (5–95 percentiles), or %.

aAdjusted to average specific gravity of 1.012 g/ml.

bGW14, n = 132.

cGW14, n = 122.

dGW14; n = 488.

GW8 (P < .001; Figure 1), and the differences were less obvious at the lower exposure levels (U-As < 58μg/l with exposure mainly through food). Also, the mothers’ U-As was inversely correlated with their urinary %DMA and positively correlated with their %iAs and %MMA. The corresponding correlations in the

**Table 2** Spearman Correlation Coefficients (P-value) for Arsenic Metabolites in Pregnancy and at 9 Years (n = 488)

| GW8       | % iAs    | %MMA    | %DMA    | U-As    | 9 years | % iAs    | %MMA    | %DMA    | U-As    |
|-----------|----------|----------|---------|---------|---------|----------|----------|---------|---------|
| %iAs      | 0.36 (<.001) | −0.91 (<.001) | −0.67 (<.001) | 0.21 (<.001) | 0.26 (<.001) | −0.28 (<.001) |<|<|<|<|
| %MMA      | 0.17 (<.001) | 0.14 (0.001) | 0.12 (0.001) | 0.12 (0.001) | 0.31 (<.001) | 0.36 (<.001) | 0.14 (0.0025) | 0.19 (<.0003) | −0.021 (0.96) | 0.96 |
| %DMA      | 0.15 (<.001) | 0.12 (0.0063) | 0.18 (<.001) | 0.064 (0.16) | −0.064 (<.001) | 0.36 (<.001) | 0.14 (0.0025) | 0.19 (<.0003) | −0.021 (0.96) | 0.96 |
| U-As      | 0.14 (0.0018) | 0.14 (0.0015) | 0.37 (<.001) | 0.018 (0.69) | 0.23 (<.0003) | 0.19 (<.0003) | 0.18 (<.0003) | 0.22 (<.0001) | 0.12 (0.0059) | 0.79 |
| Ery-As    | 0.053 (0.24) | 0.025 (0.58) | −0.063 (0.16) | 0.029 (0.53) | 0.19 (<.0001) | 0.18 (<.0001) | 0.22 (<.0001) | 0.12 (0.0059) | 0.79 |
| Folate    | −0.029 (0.39) | −0.0081 (0.86) | 0.039 (0.39) | −0.027 (0.56) | −0.15 (<.0001) | −0.0067 (0.88) | 0.093 (0.041) | 0.017 (0.71) |

**Abbreviations:** DMA, dimethylarsinic acid; Ery-As, erythrocyte arsenic; Ery-Cu, erythrocyte copper; Ery-Mn, erythrocyte manganese; Ery-Se, erythrocyte selenium; GW, gestational week; iAs, inorganic arsenic; MMA, methylarsonic acid; U-As, urinary arsenic; U-Se, urinary selenium.
Stratifying by U-As at 58 µg/l showed that U-As (+0.78 pp per 10 µg/l; \( P = 0.005 \)) and season of sampling (−4.0 pp at monsoon compared to pre-monsoon; \( P < .001 \)) were strong influencing factors mainly in the low U-As stratum, while Ery-Se was the strongest influencing factor above 58 µg/l (−4.1 pp from 5th to 95th percentile of Ery-Se; \( P < .001 \); Supplementary Table S4).

In the sensitivity analyses, the addition of maternal U-As and U-Se at GW8 to all the models had no large impact on any of the factors associated with children’s %iAs, %MMA or %DMA, and neither maternal U-As nor U-Se were by themselves associated with any of the children’s metabolites. Replacing U-As in the models with Ery-As showed that this was associated with all metabolites in the same directions and similar strengths as U-As (data not shown). The Dirichlet regression model showed similar results as the linear regression models for each metabolite (Supplementary Table S5).

Additionally, we assessed the factors influencing child U-As and Ery-As (Table 4). This showed that U-As was significantly higher at monsoon season of sampling (+54 µg/l; \( P < .001 \)) compared to pre-monsoon, and positively associated with Ery-Se (5–95th percentile: +41 µg/l; \( P = .026 \); corresponding to 0.3 SD), whereas maternal age was inversely associated with child U-As (\( B = −2.4 \mu g/l \) per year; 95% CI: −4.3, −0.46; \( P = .015 \)). For Ery-As, the influencing factors were even more obvious than for U-As (Table 4).

The difference between the concentration of U-As\textsubscript{tot} (all forms of arsenic) and U-As (iAs = MMA + DMA), observed for some of the children, was not explained by arsenobetaine, as we were unable to detect this in a subsample of children (\( n = 20 \)) from the same study population with a high ratio of U-As\textsubscript{tot}/U-As. There appeared to be more children with larger difference between U-As\textsubscript{tot} and U-As in the highest quartile of U-Se (range 18–55 µg/l) compared to the lowest (range 2.2–11 µg/l; mean difference 5.7 µg/l vs 0.53 µg/l in highest and lowest quartile of U-Se, respectively; \( P = .0024 \)), and the overall positive correlation between U-Se and the ratio U-As\textsubscript{tot}/U-As was significant, but weak (\( r_s = 0.16, P < .001 \)).

**DISCUSSION**

This study suggests that children’s metabolism of iAs differs from that in adults, which might explain the lack of data on arsenic metabolism as a susceptibility factor for arsenic toxicity in children. The main influencing factor for the urinary arsenic metabolites at 9 years of age was the selenium status, assessed as Ery-Se, which unexpectedly was associated with increasing %iAs and %MMA, but decreasing %DMA. This was particularly obvious at higher arsenic exposure levels, where the influence from ingested DMA (with the rice-based diet) had little impact on the urinary metabolite pattern. Plasma folate was associated with lower %iAs and higher %DMA. Factors of minor importance for the children’s arsenic methylation included the maternal arsenic metabolite pattern (suggesting genetic predisposition), and maternal education and SES (for %MMA only). Other nutritional markers (zinc, copper, and manganese, Hb, vitamin B12, and child anthropometry) had no apparent impact, and neither had children’s age or gender.

The children had higher %DMA and lower %iAs compared to their mothers during pregnancy, as found at younger age (Fangstrom et al., 2009). As the %DMA increases during pregnancy (Gardner et al., 2011b), the differences are even more pronounced when compared to adults in general (Hall et al., 2009; Lindberg et al., 2008a; Sun et al., 2007). Possibly, the number of factors impairing the methylation of iAs to DMA increases with...
Table 3 Multivariable-Adjusted Linear Regression Analyses of Children’s % Inorganic Arsenic (iAs), % Methylarsonic Acid (MMA), % Dimethylarsinic Acid (DMA), and Influencing Factors (n = 488)

| Influencing Factors       | %iAs P value | %MMA P value | %DMA P value |
|---------------------------|--------------|--------------|--------------|
| Age (years)               | –0.96 (–3.8, 1.9) | 0.51 | 2.0 (–0.78, 4.8) | 0.16 | –0.77 (–5.2, 3.7) | 0.73 |
| Stunting                  | 0.065 (–0.66, 0.79) | 0.86 | 0.30 (–0.40, 1.0) | 0.40 | –0.41 (–1.5, 0.70) | 0.47 |
| Pre-monsoon               | Reference     | Reference     | Reference     |
| Monsoon                   | 2.1 (1.2, 2.9) | <0.001 | 0.95 (0.14, 1.8) | 0.022 | –3.1 (–4.4, –1.8) | <0.001 |
| Post-monsoon              | 0.87 (–0.086, 0.26) | 0.33 | 0.47 (–0.30, 1.2) | 0.23 | –1.3 (–2.5, –0.11) | 0.033 |
| U-As < 58 µg/l            | –0.066 (–0.088, –0.044) | <0.001 | 0.016 (–0.053, 0.037) | 0.14 | 0.052 (0.019, 0.086) | 0.002 |
| U-As ≥ 58 µg/l            | –0.00041 (–0.0033, 0.0024) | 0.78 | 0.0046 (0.0018, 0.0073) | 0.001 | –0.0043 (–0.0087, 0.00014) | 0.058 |
| U-Se (µg/g Hb)            | –0.11 (–0.16, –0.058) | <0.001 | 0.0037 (–0.046, 0.053) | 0.88 | 0.11 (0.028, 0.19) | 0.008 |
| Ery-As (µg/g Hb)          | 7.8 (3.5, 12) | <0.001 | 6.3 (2.1, 10) | 0.003 | –14 (–21, –7.2) | <0.001 |
| Folate (µg/l)             | –0.22 (–0.35, –0.098) | <0.001 | –0.045 (–0.16, 0.075) | 0.46 | 0.26 (0.067, 0.45) | 0.008 |
| %metabolite GW8           | 0.050 (0.018, 0.083) | 0.002 | 0.10 (0.030, 0.18) | 0.006 | 0.081 (0.038, 0.12) | <0.001 |
| SES GW8                   | 0.087 (–0.086, 0.26) | 0.33 | 0.19 (0.027, 0.36) | 0.023 | –0.28 (–0.55, –0.014) | 0.039 |
| Maternal education (years)| –0.042 (–0.14, 0.052) | 0.38 | 0.16 (0.066, 0.25) | 0.001 | –0.12 (–0.26, 0.029) | 0.12 |
| Maternal age (years)      | 0.018 (–0.036, 0.071) | 0.52 | 0.054 (0.0026, 0.10) | 0.039 | –0.073 (–0.15, 0.0089) | 0.081 |

Estimates are shown as β (95% CI).
Abbreviations: iAs, inorganic arsenic; DMA, dimethylarsinic acid; Ery-Se, erythrocyte selenium; MMA, methylarsonic acid; SES, Socioeconomic status; U-As, Urinary arsenic; U-Se, urinary selenium.

Table 4 Multivariable-Adjusted Linear Regression Analyses of Children’s U-As and Ery-As, and Influencing Factors (n = 488)

| Influencing factors | U-As P-value | Ery-As P-value |
|---------------------|--------------|---------------|
| Age (years)         | 85 (–18, 188) | 0.11 | 3.3 (–1.5, 8.1) | 0.17 |
| Stunting            | –12 (–38, 14) | 0.37 | –0.55 (–1.8, 0.66) | 0.38 |
| Pre-monsoon         | Reference     | Reference     |
| Monsoon             | 54 (24, 84) | <0.001 | 2.7 (1.4, 4.1) | <0.001 |
| Post-monsoon        | 7.9 (–20, 36) | 0.58 | 0.33 (–0.99, 1.7) | 0.62 |
| U-Se (µg/l)         | –1.4 (–3.2, 0.46) | 0.14 | –0.13 (–0.21, –0.044) | 0.003 |
| Ery-Se (µg/g Hb)    | 177 (22, 333) | 0.026 | 13 (5.4, 20) | 0.001 |
| Folate (µg/l)       | –2.0 (–6.5, 2.4) | 0.37 | –0.15 (–0.36, 0.053) | 0.15 |
| SES GW8             | –0.21 (–6.5, 6.0) | 0.95 | –0.020 (–0.31, 0.27) | 0.89 |
| Maternal education (years) | –2.6 (–5.9, 0.79) | 0.13 | –0.16 (–0.31, 0.00040) | 0.050 |
| Maternal age (years) | –2.4 (–4.3, –0.46) | 0.015 | –0.11 (–0.20, –0.027) | 0.011 |

Estimates are shown as β (95% CI).
Abbreviations: Ery-As, erythrocyte arsenic; Ery-Se, erythrocyte selenium; MMA, methylarsonic acid; GW, gestational week; SES, Socioeconomic status; U-As, urinary arsenic; U-Se, urinary selenium.

Age (Lindberg et al., 2008a), such as smoking (Lindberg et al., 2010) and decreased liver function (Schmucker, 2005). Also, the fact that the metabolite pattern in the children was not markedly influenced by higher exposure levels indicates that their methylation of arsenic is not as sensitive to inhibition as that in adults. For the %MMA, the main susceptibility factor for arsenic toxicity in adults (Lindberg et al., 2008b), the increase in %MMA was 0.46 pp MMA per 100 µg/l in U-As for the presently studied children, compared to 0.78 pp MMA/100 µg/l in adults in the same study area (Lindberg et al., 2008a). A previous study on children of the same study population found a positive association between higher %MMA and growth (Gardner et al., 2013), and another study found no impact of %MMA on the inverse associations between arsenic exposure and children’s neurodevelopment (Hamadani et al., 2011), supporting that %MMA is not a measure of susceptibility to arsenic-induced toxicity. There was no difference in the methylation pattern induced by boys and girls, suggesting hormonal influences later in life (Lindberg et al., 2008a).

The positive associations between Ery-Se and %As and %MMA, and inverse association with %DMA, all appeared linear and robust towards additional adjustments. The results are in contrast to previous findings in the mothers (Li et al., 2008) and other adults (Hsueh et al., 2003; Pfinsler et al., 2011), in whom the arsenic metabolite pattern in urine was not influenced by plasma or serum selenium. To note, Ery-Se is considered a more long-term marker of the selenium status than is plasma selenium (Thomson, 2004). The observed associations might be related to competition for methyl groups and glutathione, both involved in the 1-carbon metabolism. Similar to arsenic, selenium is methylated to selenosugars and the trimethylselenonium ion, which are excreted via urine ( Francesconi and Pannier, 2004; Jackson et al., 2013; Kuehnelt et al., 2015). One hypothesis is therefore that a poor 1-carbon metabolism would result in lower selenium excretion and higher Ery-Se, as well as lower %DMA. An efficient 1-carbon metabolism and availability of methyl groups is likely particularly important in growing children to meet the demands for production of e.g. creatine, proteins, phospholipids, and DNA, which might explain the particularly strong association between blood selenium and arsenic metabolism in the children. Another explanation for the inverse association between Ery-Se and %DMA could be that selenium inhibits arsenic methylation through binding to and modification of AS3MT, the main arsenic methyltransferase (Geng et al., 2009). In primary human hepatocytes, the inhibition of arsenic methylation seemed to depend on the selenium species;
selenite exhibited the strongest effect, and this was obvious already at sub-μM concentrations (Walton et al., 2003).

Surprisingly, the urinary excretion of arsenic increased with increasing Ery-Se, despite the fact that Ery-Se was associated with lower %DMA in urine. In adults, a higher %DMA is usually associated with a higher rate of overall arsenic excretion up to a certain exposure level where the arsenic starts inhibiting its own methylation (Vahter, 2002). An increase from the 5th to the 95th percentile in the children’s Ery-Se corresponded to 41 μg/l higher U-As, i.e., quite a substantial change considering that the median U-As was 53 μg/l. On the other hand, Ery-Se showed a strong positive association also with Ery-As (5–95th increase in Ery-Se: +3 μg/kg in Ery-As), why reverse causality cannot be excluded, i.e. that elevated arsenic exposure (high U-As and Ery-As) might alter the selenium kinetics. However, a study in Bangladeshi adults (n = 287) with quite high arsenic exposure through drinking water found a strong inverse association between plasma selenium (mean 87.6 g/l) and total arsenic in urine (mean 173 μg/l; but no association with the urinary arsenic metabolites) and a weaker inverse association with blood arsenic [at the highest plasma selenium only] (Pilsner et al., 2011). Another study from the same study area found much weaker inverse associations of blood selenium with total arsenic metabolites (total arsenic) and an inverse association with %DMA, which in contrast to certain experimental animals (Anundi et al., 2000), and freshwater fish (most likely the type of fish consumed in the present area) contains less arsenobetaine compared to marine fish (Caumette et al., 2012).

The association between maternal and child metabolite pattern is in line with a genetic influence on the metabolism of arsenic, and previous studies have also shown a family-related arsenic metabolite pattern (Chung et al., 2002; Tellez-Plaza et al., 2013). Arsenic methylation rate is influenced by single nucleotide polymorphisms (SNPs) in the gene encoding for AS3MT, and our previous studies showed that the genotype associated with less efficient arsenic methylation is fairly prevalent among the studied mothers (Engstrom et al., 2013). In a previous study, the arsenic metabolite patterns seemed more similar between siblings than between parents and their children (Chung et al., 2002), which is also in line with the present findings of differences in methylation efficiency between children and adults. Also, the source of arsenic seems to affect the metabolite pattern. At fairly low exposure levels (<5 μg/l in urine), when the ingested arsenic is largely from food (Vahter et al., 2006), %As decreased and %DMA increased with increasing exposure, probably because a part of arsenic in rice is present as DMA (Meharg et al., 2008). Indeed, increasing intake of rice has been found to increase the %DMA in urine (deCastro et al., 2014; Kordas et al., 2016). This may also explain why the highest %DMA appeared during the pre-monsoon period, with higher food security (Hillbrunner and Egan, 2008). The highest U-As appeared during the monsoon period, possibly due to higher water arsenic concentrations (Bhattacharya et al., 2011) and/or increased use of nearby, shallow wells.

The strengths of our study include the relatively large study sample (n = 488 mother–child pairs) and the longitudinal design. Also, biomarkers of arsenic and selenium status were analyzed by ICPMS, and we had the possibility to compare different media for each element. Limitations include lack of plasma selenium to assess selenium kinetics, no data regarding other nutrients known to affect the methylation of arsenic, such as homocysteine and vitamin B6, involved in the 1-carbon metabolism, and lack of AS3MT genotype. Also, functional markers of selenium status such as glutathione peroxidases or thioredoxin reductases could have provided information regarding their impact on the arsenic metabolism through the provision of reducing equivalents necessary for the reduction of iAs and its metabolites (Burk and Levander, 2006).

To conclude, children had higher arsenic methylation efficiency than adults, and there was no difference between boys and girls. The main influencing factors were selenium and folate status. The strong influence of selenium is in contrast to that observed in adults, and the mechanisms of the interaction need to be further elucidated. Minor influencing factors were genetic predisposition, SES, and ongoing exposure to arsenic.

The positive association of total U-Se excretion with %DMA, and inverse association with %iAs, were similar to findings in the mothers (unpublished data) and other adults (Hsueh et al., 2003). The association might be explained by coexcretion of methylated metabolites of arsenic and selenium, and, as mentioned above, a similar influence from 1-carbon metabolism. Previously, a similar positive correlation has been observed for %DMA and urinary creatinine, which is produced from creatine, a major product of humans, although such a complex has never been identified in human urine. We found that more U-As (about 10%) in other form(s) than the measured arsenic metabolites (total arsenic minus sum of iAs metabolites) was excreted in the highest quartile of U-Se compared to the lowest, possibly suggesting the excretion of an arsenic-selenium complex. As we were unable to detect arsenobetaine in a subsample of children from the same study population (with high ratio total arsenic/sum of metabolites) the difference could not be due to co-excretion of selenium and organic arsenicals commonly occurring in seafood. As we have not explored the potential presence of a selenium–arsenic complex, its presence in the children’s urine is merely speculations that need to be addressed further. The absence of arsenobetaine in the children’s urine is consistent with a relatively low consumption of fish (37–55 g/d in children living in similar areas (Khan et al., 2009)). Also, fish collected from a nearby village contained only trace amounts of arsenic (Das et al., 2004), and freshwater fish (most likely the type of fish consumed in the present area) contains less arsenobetaine compared to marine fish (Caumette et al., 2012).
The results emphasize the need for considering the dietary intake of different arsenic species when evaluating the arsenic metabolite pattern in urine.

SUPPLEMENTARY DATA
Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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References
Ahmed, S., Ahsan, K. B., Kippler, M., Mily, A., Wagatsuma, Y., Hoque, A. M., Ngom, P. T., El Arifeen, S., Raqib, R., and Vahter, M. (2012). In utero arsenic exposure is associated with impaired thymic function in newborns possibly via oxidative stress and apoptosis. Toxicol. Sci. 129, 305–314.
Ahmed, S., Moore, S. E., Kippler, M., Gardner, R., Hawlader, M. D., Wagatsuma, Y., Raqib, R., and Vahter, M. (2014). Arsenic exposure and cell-mediated immunity in preschool children in rural Bangladesh. Toxicol. Sci. 141, 166–175.
Ahmed, S., Rekha, R. S., Ahsan, K. B., Doi, M., Grander, M., Roy, A., K., Ekstrom, E. C., Wagatsuma, Y., Vahter, M., and Raqib, R. (2013). Arsenic exposure affects plasma insulin-like growth factor 1 (IGF-1) in children in rural Bangladesh. PLoS One 8, e81530.
Antonelli, R., Shao, K., Thomas, D. J., Sams, R., and 2nd, Cowden, J. (2014). AS3MT, GSTO, and PNP polymorphisms: Impact on arsenic methylation and implications for disease susceptibility. Environ. Res. 132C, 156–167.
Anundi, I., Hogberg, J., and Vahter, M. (1982). GSH release in bile as influenced by arsenite. FEBS Lett. 145, 285–288.
Bailey, L. B., Stover, P. J., McNulty, H., Fenech, M. F., Gregory, J. F., 3rd, Mills, J. L., Pfeiffer, C. M., Fazili, Z., Zhang, M., et al. (2015). Biomarkers of Nutrition for Development-Folate Review. J. Nutr. 145, 1636S–1680S.
Berry, J. P., and Galle, P. (1994). Selenium-arsenic interaction in renal cells: role of lysosomes. Electron microprobe study. J. Submicrosc. Cytol. Pathol. 26, 203–210.
Bhattacharya, P., Hossain, M., Rahman, S. N., Robinson, C., Nath, B., Rahman, M., Islam, M. M., Von Bromssen, M., Ahmed, K. M., Jacks, G., et al. (2011). Temporal and seasonal variability of arsenic in drinking water wells in Matlab, southeastern Bangladesh: a preliminary evaluation on the basis of a 4 year study. J. Environ. Sci. Health A Tox Hazard Subst. Environ. Eng. 46, 1177–1184.
Burk, R. F., and Levander, O. A. (2006). Selenium. In Modern Nutrition in Health and Disease (Shils MF MS, Ross C, Caballero B, and C. RJ, Eds.). Lippincott Williams & Wilkins, Philadelphia.
Carew, M. W., and Leslie, E. M. (2010). Selenium-dependent and independent transport of arsenic by the human multidrug resistance protein 2 (MRP2/ABCC2): implications for the mutual detoxification of arsenic and selenium. Carcinogenesis 31, 1450–1455.
Caumette, G., Koch, I., and Reimer, K. J. (2012). Arsenobetaine formation in plankton: a review of studies at the base of the aquatic food chain. JEM 14, 2841–2853.
Chen, C. J., Hsu, L. I., Wang, C. H., Shih, W. L., Hsu, Y. H., Tseng, M. P., Lin, Y. C., Chou, W. L., Chen, C. Y., Lee, C. Y., et al. (2005). Biomarkers of exposure, effect, and susceptibility of arsenic-induced health hazards in Taiwan. Toxicol. Appl. Pharmacol. 206, 198–206.
Chung, J. S., Kalman, D. A., Moore, L. E., Kosnett, M. J., Arroyo, A. P., Beeris, M., Mazumder, D. N., Hernandez, A. L., and Smith, A. H. (2002). Family correlations of arsenic methylation patterns in children and parents exposed to high concentrations of arsenic in drinking water. Environ. Health Perspect. 110, 729–733.
Das, H. K., Mitra, A. K., Sengupta, P. K., Hossain, A., Islam, F., and Rabban, G. H. (2004). Arsenic concentrations in rice, vegetables, and fish in Bangladesh: a preliminary study. Environ. Int. 30, 383–387.
deCastro, B. R., Caldwell, K. L., Jones, R. L., Blount, B. C., Pan, Y., Ward, C., and Mortensen, M. E. (2014). Dietary sources of methylated arsenic species in urine of the United States population, NHANES 2003–2010. PLoS One 9, e108098.
Engstrom, K., Vahter, M., Miakar, S. J., Concha, G., Nermell, B., Raqib, R., Cardozo, A., and Broberg, K. (2011). Polymorphisms in arsenic(+III oxidation state) methyltransferase (AS3MT) predict gene expression of AS3MT as well as arsenic metabolism. Environ. Health Perspect. 119, 182–188.
Engstrom, K. S., Hossain, M. B., Lauss, M., Ahmed, S., Raqib, R., Vahter, M., and Broberg, K. (2013). Efficient arsenic metabolism–the AS3MT haplotype is associated with DNA methylation and expression of multiple genes around AS3MT. PLoS One 8, e53732.
Faita, F., Cori, L., Bianchi, F., and Andreassi, M. G. (2013). Arsenic-induced genotoxicity and genetic susceptibility to arsenic-related pathologies. Int. J. Environ. Res. Public Health 10, 1527–1546.
Fangstrom, B., Hamadan, J., Nermell, B., Grander, M., Palm, B., and Vahter, M. (2009). Impaired arsenic metabolism in children during weaning. Toxicol. Appl. Pharmacol. 239, 208–214.
Fangstrom, B., Moore, S., Nermell, B., Kuenstl, L., Goessler, W., Grander, M., Kabir, I., Palm, B., Arifeen, S. E., and Vahter, M. (2008). Breast-feeding protects against arsenic exposure in Bangladeshi infants. Environ. Health Perspect. 116, 963–969.
Francesconi, K. A., and Pannier, F. (2004). Selenium metabolites in urine: a critical overview of past work and current status. Clin. Chem. 50, 2240–2253.
Gardner, R., Hamadan, J., Grander, M., Tofail, F., Nermell, B., Palm, B., Kippler, M., and Vahter, M. (2011a). Persistent exposure to arsenic via drinking water in rural Bangladesh despite major mitigation efforts. Am. J. Public Health 101 Suppl 1, S333–S338.
Gardner, R. M., Engstrom, K., Bottai, M., Hoque, W. A., Raqib, R., Broberg, K., and Vahter, M. (2012). Pregnancy and the methyltransferase genotype independently influence the arsenic methylation phenotype. Pharmacogenet. Genomics 22, 508–516.

Gardner, R. M., Kippler, M., Tofail, F., Bottai, M., Hamadani, J., Grander, M., Nermell, B., Palm, B., Rasmussen, K. M., and Vahter, M. (2013). Environmental exposure to metals and children’s growth to age 5 years: a prospective cohort study. Am. J. Epidemiol. 177, 1536–1547.

Gardner, R. M., Nermell, B., Kippler, M., Grander, M., Li, L., Ekstrom, E. C., Rahman, A., Lonnerdal, B., Hoque, A. M., and Vahter, M. (2011b). Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status. Reprod. Toxicol. 31, 210–218.

Geng, Z., Song, X., Xing, Z., Geng, J., Zhang, S., Zhang, X., and Wang, Z. (2009). Effects of selenium on the structure and function of recombinant human S-adenosyl-L-methionine dependent arsenic (±3 oxidation state) methyltransferase in col.I. Biol. Inorg. Chem. 14, 485–496.

George, C. M., Gamble, M., Slavkovich, V., Levy, D., Ahmed, A., Ahsan, H., and Graziano, J. (2013). A cross-sectional study of the impact of blood selenium on blood and urinary arsenic concentrations in Bangladesh. Environ. Health 12, 52.

Hall, M. N., Liu, X., Slavkovich, V., Ilievski, V., Pilsner, J. R., Alam, S., Factor-Litvak, P., Graziano, J. H., and Gamble, M. V. (2009). Folate, Cobalamin, Cysteine, Homocysteine, and Arsenic Metabolism among Children in Bangladesh. Environ. Health Perspect. 117, 825–831.

Hamadani, J. D., Tofail, F., Nermell, B., Gardner, R., Shiraji, S., Bottai, M., Arifeen, S. E., Huda, S. N., and Vahter, M. (2011). Critical windows of exposure for arsenic-associated impairment of cognitive function in pre-school girls and boys: a population-based cohort study. Int. J. Epidemiol. 40, 1593–1604.

Hillbrunner, C., and Egan, R. (2008). Seasonality, household food security, and nutritional status in Dinajpur, Bangladesh. Food Nutr. Bull. 29, 221–231.

Howe, C. G., Niedzwiecki, M. M., Hall, M. N., Liu, X., Ilievski, V., Slavkovich, V., Alam, S., Siddique, A. B., Graziano, J. H., and Gamble, M. V. (2014). Folate and cobalamin modify associations between S-adenosylmethionine and methylated arsenic metabolites in arsenic-exposed Bangladeshi adults. J. Nutr. 144, 690–697.

Hsueh, Y. M., Ko, Y. F., Huang, Y. K., Chen, H. W., Chioy, H. Y., Huang, Y. I., Yang, M. H., and Chen, C. J. (2003). Determinants of inorganic arsenic methylation capability among residents of the Lanyang Basin, Taiwan: arsenic and selenium exposure and alcohol consumption. Toxicol. Lett. 137, 49–63.

Huang, Z., Pei, Q., Sun, G., Zhang, S., Liang, J., Gao, Y., and Zhang, X. (2008). Low selenium status affects arsenic metabolites in an arsenic exposed population with skin lesions. Clin. Chim. Acta 387, 139–144.

IARC. (2012). A review of human carcinogens: Arsenic, metals, fibres, and dusts. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, International Agency for Research on Cancer, World Health Organization, Lyon, France.[TQ1]

Jackson, M. I., Lunoe, K., Gabel-Jensen, C., Gammelgaard, B., and Combs, G. F. Jr. (2013). Metabolism of selenite to selenosugar and trimethylselenonium in vivo: tissue dependency and requirement for S-adenosylmethionine-dependent methylation. J. Nutr. Biochem. 24, 2023–2030.

Kala, S. V., Kala, G., Prater, C. I., Sartorelli, A. C., and Lieberman, M. W. (2004). Formation and urinary excretion of arsenic triglutathione and methylarsenic diglutathione. Chem. Res. Toxicol. 17, 243–249.

Kala, S. V., Neely, M. W., Kala, G., Prater, C. I., Atwood, D. W., Rice, J. S., and Lieberman, M. W. (2000). The MRP2/cMOAT transporter and arsenic-glutathione complex formation are required for biliary excretion of arsenic. J. Biol. Chem. 275, 33404–33408.

Khan, N. I., Bruce, D., Naidu, R., and Owens, G. (2009). Implementation of food frequency questionnaire for the assessment of total dietary arsenic intake in Bangladesh: part B, preliminary findings. Environ. Geochim. Health 31, 221–238.

Kippler, M., Skroder, H., Rahman, S. M., Tofail, F., and Vahter, M. (2016). Elevated childhood exposure to arsenic despite reduced drinking water concentrations - A longitudinal cohort study in rural Bangladesh. Environ. Int. 86, 119–125.

Kordas, K., Queirolo, E. I., Manay, N., Peregalli, F., Hsiao, P. Y., Lu, Y., and Vahter, M. (2016). Low-level arsenic exposure: Nutritional and dietary predictors in first-grade Uruguayan children. Environ. Res. 147, 16–23.

Kuehnelt, D., Engstrom, K., Skroder, H., Kokarnig, S., Schlebusch, C., Kippler, M., Alhamdow, A., Nermell, B., Francesconi, K., Broberg, K., and., et. al. (2015). Selenium metabolism to the trimethylselenonium ion (TMSe) varies markedly because of polymorphisms in the indolethylamine N-methyltransferase gene. Am. J. Clin. Nutr. 102, 1406–1415.

Levander, O. A. (1977). Metabolic interrelationships between arsenic and selenium. Environ. Health Perspect. 19, 159–164.

Li, L., Ekstrom, E. C., Goessler, W., Lonnerdal, B., Nermell, B., Yunus, M., Rahman, A., El Arifeen, S., Persson, L. A., and Vahter, M. (2008). Nutritional status has marginal influence on the metabolism of inorganic arsenic in pregnant Bangladeshi women. Environ. Health Perspect. 116, 315–321.

Lindberg, A. L., Ekstrom, E. C., Nermell, B., Rahman, M., Lonnerdal, B., Persson, L. A., and Vahter, M. (2008a). Gender and age differences in the metabolism of inorganic arsenic in a highly exposed population in Bangladesh. Environ. Res. 106, 110–120.

Lindberg, A. L., Rahman, M., Persson, L. A., and Vahter, M. (2008b). The risk of arsenic induced skin lesions in Bangladeshi men and women is affected by arsenic metabolism and the age at first exposure. Toxicol. Appl. Pharmacol. 230, 9–16.

Lindberg, A. L., Sohel, N., Rahman, M., Persson, L. A., and Vahter, M. (2010). Impact of smoking and chewing tobacco on arsenic-induced skin lesions. Environ. Health Perspect. 118, 533–538.

Lu, Y., Kippler, M., Harari, F., Grander, M., Palm, B., Nordqvist, H., and Vahter, M. (2015). Alkali dilution of blood samples for high throughput ICP-MS analysis-comparison with acid digestion. Clin. Biochem. 48, 140–147.

Marafante, E., Vahter, M. (1984). The effect of methyltransferase inhibition on the metabolism of [74As]arsenite in mice and rabbits. Chem. Biol. Interact. 50, 49–57.

Meharg, A. A., Lombi, E., Scheckel, K. G., Feldmann, J., Raab, A., Zhu, Y., and Islam, R. (2008). Speciation and localization of arsenic in white and brown rice grains. Environ. Sci. Technol. 42, 1051–1057.

Nermell, B., Lindberg, A. L., Rahman, M., Berglund, M., Persson, L. A., El Arifeen, S., and Vahter, M. (2008). Urinary arsenic concentration adjustment factors and malnutrition. Environ. Res. 106, 212–218.

Persson, L. A., Arifeen, S., Ekstrom, E. C., Rasmussen, K. M., Frongillo, E. A., Yunus, M., and Team, M. I. S. (2012). Effects of prenatal micronutrient and early food supplementation on maternal hemoglobin, birth weight, and infant mortality.
among children in Bangladesh: the MINIMat randomized trial. JAMA 307,
Peters, B. A., Hall, M. N., Liu, X., Neugut, Y. D., Pilsner, J. R., Levy, D., Ilievski, V., Slavkovich, V., Islam, T., Factor-Litvak, P., et al. (2014). Creatinine, arsenic metabolism, and renal function in an arsenic-exposed population in Bangladesh. PloS One 9, e113760.
Pilsner, J. R., Hall, M. N., Liu, X., Ahsan, H., Ilievski, V., Slavkovich, V., Levy, D., Factor-Litvak, P., Graziano, J. H., and Gamble, M. V. (2011). Associations of plasma selenium with arsenic and genomic methylation of leukocyte DNA in Bangladesh. Environ. Health Perspect. 119, 113–118.
Schmucker, D. L. (2005). Age-related changes in liver structure and function: Implications for disease? Exp. Gerontol. 40, 650–659.
Skröder, H. M., Hamadani, J. D., Tofail, F., Persson, L., Vahter, M. E., and Kippler, M. J. (2015). Selenium status in pregnancy influences children’s cognitive function at 1.5 years of age. Clin. Nutr. 34, 923–930.
Smith, A. H., Marshall, G., Liaw, J., Yuan, Y., Ferreccio, C., and Steinmaus, C. (2012). Mortality in young adults following in utero and childhood exposure to arsenic in drinking water. Environ. Health Perspect. 120, 1527–1531.
Smith, A. H., Yunus, M., Khan, A. F., Ercumen, A., Yuan, Y., Smith, M. H., Liaw, J., Balmes, J., von Ehrenstein, O., Raqib, R., et al. (2013). Chronic respiratory symptoms in children following in utero and early life exposure to arsenic in drinking water in Bangladesh. Int. J. Epidemiol. 42, 1077–1086.
Soto-Pena, G. A., Luna, A. L., Acosta-Saavedra, L., Conde, P., Lopez-Carrillo, L., Cebrian, M. E., Bastida, M., Calderon-Aranda, E. S., and Vega, L. (2006). Assessment of lymphocyte subpopulations and cytokine secretion in children exposed to arsenic. Faseb J. 20, 779–781.
Stefanowicz, F. A., Talwar, D., O’Reilly, D. S., Dickinson, N., Atkinson, J., Hurshhouse, A. S., Rankin, J., and Duncan, A. (2013). Erythrocyte selenium concentration as a marker of selenium status. Clin. Nutr. 32, 837–842.
Sun, G., Xu, Y., Li, X., Jin, Y., Li, B., and Sun, X. (2007). Urinary arsenic metabolites in children and adults exposed to arsenic in drinking water in Inner Mongolia, China. Environ. Health Perspect. 115, 648–652.
Tellez-Plaza, M., Griebble, M. O., Voruganti, V. S., Francesconi, K. A., Goessler, W., Umans, J. G., Silbergeld, E. K., Guallar, E., Franceschini, N., North, K. E., et al. (2013). Heritability and preliminary genome-wide linkage analysis of arsenic metabolites in urine. Environ. Health Perspect. 121, 345–351.
Thomson, C. D. (2004). Assessment of requirements for selenium and adequacy of selenium status: a review. Eur. J. Clin. Nutr. 58, 391–402.
Tseng, C. H. (2009). A review on environmental factors regulating arsenic methylation in humans. Toxicol. Appl. Pharmacol. 235, 338–350.
Vahter, M. (2002). Mechanisms of arsenic biotransformation. Toxicology 181-182, 211–217.
Vahter, M. E., Li, L., Nermell, B., Rahman, A., El Arifeen, S., Rahman, M., Persson, L. A., and Ekstrom, E. C. (2006). Arsenic exposure in pregnancy: a population-based study in Matlab, Bangladesh. J. Health Popul. Nutr. 24, 236–245.
Walton, F. S., Waters, S. B., Jolley, S. L., LeCluyse, E. L., Thomas, D. J., and Styblo, M. (2003). Selenium compounds modulate the activity of recombinant rat AsIII-methyltransferase and the methylation of arsenite by rat and human hepatocytes. Chem. Res. Toxicol. 16, 261–265.
Wasserman, G. A., Liu, X., Parvez, F., Ahsan, H., Factor-Litvak, P., Kline, J., van Geen, A., Slavkovich, V., Loiacono, N. J., Levy, D., et al. (2007). Water arsenic exposure and intellectual function in 6-year-old children in Araihazar, Bangladesh. Environ. Health Perspect. 115, 285–289.
Zeng, H., Uthus, E. O., and Combs, G. F. Jr. (2005). Mechanistic aspects of the interaction between selenium and arsenic. J. Inorg. Biochem. 99, 1269–1274.