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Interaction of plasma glutathione redox and folate deficiency on arsenic methylation capacity in Bangladeshi adults

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Abstract

Inorganic arsenic (InAs) is metabolized through a series of methylation reactions catalyzed by arsenic(III)-methyltransferase (AS3MT), resulting in the generation of monomethylarsonic (MMAs) and dimethylarsinic acids (DMAs). AS3MT activity requires the presence of the methyl donor *S*-adenosylmethionine (SAM), a product of folate-dependent one-carbon metabolism, and a reductant. Although glutathione (GSH), the primary endogenous antioxidant, is not required for As methylation, GSH stimulates As methylation rates *in vitro*. However, the relationship between GSH redox and As methylation capacity in humans is unknown. We wished to test the hypothesis that a more oxidized plasma GSH redox status is associated with decreased As methylation capacity, and examine whether these associations are modified by folate nutritional status. Concentrations of plasma GSH and GSSG, plasma folate, total blood As (bAs), total urinary As (uAs), and uAs metabolites were assessed in a cross-sectional study of *n* = 376 Bangladeshi adults who were chronically exposed to As in drinking water. We observed that a decreased plasma GSH/GSSG ratio (reflecting a more oxidized redox state) was significantly associated with increased urinary %MMA, decreased urinary %DMA, and increased total bAs in folate-deficient individuals (plasma folate 9.0 nmol/L). Concentrations of plasma GSH and GSSG were independently associated with increased and decreased As methylation capacity, respectively. No

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significant associations were observed in folate-sufficient individuals, and interactions by folate status were statistically significant. Our findings suggest that GSH/GSSG redox regulation might contribute to the large interindividual variation in As methylation capacity observed in human populations.

Keywords

Arsenic; arsenic methylation; Bangladesh; folate; glutathione; glutathione disulfide; oxidative stress; redox

INTRODUCTION

Over 140 million people throughout Bangladesh, India, Vietnam, Nepal, and Cambodia are chronically exposed to arsenic (As) in drinking water at concentrations over 10 μg/L, the World Health Organization guideline [1]. As a Class I human carcinogen [2], As is associated with increased risk for cancers of the skin, lung, bladder, liver, and kidney [3], although the carcinogenic mechanisms of As are incompletely understood [4].

Inorganic arsenic (InAs) is metabolized, primarily in the liver, through a series of methylation reactions catalyzed by arsenic(III)-methyltransferase (AS3MT), resulting in the formation of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) [5]. The methyl group for the reaction is donated from *S*-adenosylmethionine (SAM) [5], a product of folate-dependent one-carbon metabolism [6]. Since methylation increases As excretion and reduces As body burden [7], it is believed to be a detoxification process [8]. However, *in vitro* and *in vivo* studies have identified MMA^{III} as the most toxic As form [9, 10], suggesting that As methylation also involves bioactivation [11]. Epidemiologic studies consistently find that a reduced capacity to fully methylate InAs, as indicated by higher $\%MMA^{(III+V)}$ and lower $\%DMA^{(III+V)}$ in urine, is associated with increased risk for As-induced skin lesions and cancers of the skin, lung, and bladder [12]. As such, the identification of factors that facilitate complete methylation of InAs to DMA might provide insight into interventions to reduce risk for As-related diseases [13].

Although the proposed As methylation pathways presented in Figure 1 are distinct, they all require the presence of a reductant, such as glutathione (GSH) [14]. As the primary endogenous antioxidant, GSH readily donates an electron for reduction reactions, forming its oxidized form, glutathione disulfide (GSSG), in the process [15]. A lower ratio of GSH to GSSG reflects a more oxidized intracellular redox state [15]. While GSH is not required for As methylation to proceed, GSH can serve as the reducing agent necessary for AS3MT activity [16-19]. In addition, GSH can stimulate AS3MT-catalyzed methylation rates in experimental systems already containing another reductant [20].

GSH might facilitate As methylation by donating electrons for the reduction of pentavalent arsenate (As^{V}) to trivalent arsenite (As^{III}), shown in the oxidative methylation pathway first proposed by Challenger [21, 22] (**Figure 1A**), or by forming As-GSH complexes that are substrates for AS3MT, as proposed by Hayakawa et al. [23] (**Figure 1B**). Recent work by Wang et al. found that a reductant (such as GSH) is needed to cleave a disulfide bond within

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AS3MT, which allows As^{III} to bind the enzyme [24] (**Figure 1C**). Additionally, active-site cysteine (Cys) residues that are potentially redox-sensitive have been identified in AS3MT [25]. Redox-sensitive Cys residues are prone to oxidative modifications such as *S*-glutathionylation— the reversible formation of a mixed disulfide between a Cys residue and GSH or GSSG—which might also regulate AS3MT activity [26].

We wished to test the hypothesis that a more reduced GSH redox state is associated with increased As methylation capacity, as indicated by a decrease in urinary %MMA, increase in urinary %DMA, and increase in the urinary secondary methylation index (SMI, defined as the ratio of DMA to MMA). We measured the GSH/GSSG ratio in plasma and in blood; the plasma ratio is believed to better reflect GSH/GSSG status in liver [27], the primary site of As methylation, and was therefore chosen for our focus in the present analyses. We also examined whether the associations were modified by folate status. We used data from the Folate and Oxidative Stress (FOX) study [28], which was originally designed to assess the dose-response relationship between total As exposure and markers of oxidative stress.

SUBJECTS AND METHODS

Eligibility criteria and study design

In the FOX study, we recruited 378 men and women between the ages of 30 and 65 y between April 2007 and April 2008 in Araihazar, Bangladesh. Study participants were selected on well water As (wAs) exposure to ensure that the study sample reflected the full range of wAs exposures in the region, as previously described [28]. Participants were excluded if they were pregnant, had taken nutritional supplements within the past 3 months, or had known diabetes, cardiovascular disease, or renal disease.

Analytic techniques

Sample collection and handling—Blood samples were drawn and processed immediately at our field clinic in Araihazar. Aliquots of blood and plasma were stored at –80°C. They were then transported to Dhaka on dry ice and stored at –80°C. Samples were then shipped, frozen on dry ice, to Columbia University for analysis.

Water As—Field sample collection and laboratory procedures are described previously in detail [29, 30]. Briefly, at the time of the recruitment visit of the FOX study, new well water samples were collected in 20-mL polyethylene scintillation vials and acidified to 1% with high-purity Optima HCl (Fisher Scientific, Pittsburg, PA, USA) at least 48 hr before analysis [31]. Water samples were analyzed by high-resolution inductively coupled plasma mass spectrometry after 1:10 dilution and addition of a Ge spike to correct fluctuations in instrument sensitivity. Standards were run multiple times in each batch. The intra- and inter-assay coefficients of variation (CVs) were 3.8% and 6.0%, respectively.

Urinary As metabolites and urinary creatinine—Urinary As (uAs) metabolite species were measured using HPLC separation of arsenobetaine (AsB), arsenocholine (AsC), As^{V}, As^{III}, MMAs, and DMAs, followed by detection with ICP-MS [32]. Total uAs concentrations were determined by summing the concentrations of As^{V}, As^{III}, MMAs, and

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DMAs, excluding AsC and AsB from the sum. The limit of detection for each uAs metabolite was 0.1 μg/L. Urinary creatinine (uCr) was analyzed using a colorimetric assay based on the Jaffe reaction [33], and urinary As concentrations were calculated with and without adjustment for uCr. The intra-assay CVs were 4.5% for As^{V} , 3.8% for As^{III}, 1.5% for MMA, and 0.6% for DMA; the inter-assay CVs were 10.6% for As^{V} , 9.6% for As^{III}, 3.5% for MMA, and 2.8% for DMA.

Total blood As—Total blood As (bAs) concentrations were determined by Perkin-Elmer Elan DRC II ICPMS equipped with an AS 93+ autosampler, as previously described [34]. The limit of detection for bAs was 0.1 μg/L. The intra- and inter-assay CVs were 3.2% and 5.7%, respectively.

Plasma folate and cobalamin—Plasma folate and cobalamin were measured using a radioproteinbinding assay (SimulTRAC-S, MP Biomedicals, Orangeburg, NY). For determination of folate concentration, folic acid as pteroylglutamic acid was used for calibration, and its ^{125}I-labeled analog was used as the tracer; for cobalamin, cyanocobalamin was used for calibration, and its $_{57}$Co-labeled analog was used as the tracer. The intra- and inter-assay CVs for folate were 6% and 14%, respectively, and the intra- and inter-assay CVs for cobalamin were 4% and 8%, respectively.

Blood and plasma glutathione and glutathione disulfide—Whole blood and plasma GSH and GSSG were assayed based on the protocol by Jones et al [35], as previously described [28]. Briefly, blood was collected in the field laboratory in Bangladesh and immediately transferred into Eppendorf tubes containing 5% perchloric acid (PCA), 0.1 M boric acid, and γ-glutamyl glutamate (internal standard). Samples were derivatized in Bangladesh, and the derivatized samples were stored at –80°C until delivered to Columbia University on dry ice for HPLC analysis. Metabolites were detected using a Waters 474 scanning fluorescence detector, with 335 nm excitation and 515 nm emission (Waters Corp., Milford, MA). Intra-assay CVs were all between 5 and 10%, and inter-assay CVs were between 11 and 18%.

Calculation of the reduction potential

The reduction potential of the thiol/disulfide plasma GSH/GSSG redox pair (E_h) was calculated using the Nernst equation (E_h=E_o + RT/nF ln [disulfide]/[thiol]^2 where E_o=standard potential for the redox couple, R=gas constant, T=absolute temperature, n=2 for the number of electrons transferred, and F=Faraday's constant) [36]. A more positive E_h value reflects a more oxidized redox state.

Statistical methods

Descriptive statistics were calculated for the overall sample. Chi-square test and the Wilcoxon rank-sum test were used to detect group differences in categorical and continuous variables, respectively. Spearman correlations were used to examine bivariate associations between quantitative variables, including associations between covariates with urinary As metabolite percentages and plasma GSH and GSSG concentrations. Certain covariates (gender, age, ever cigarette smoking, and water As) were selected based on biologic

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plausibility and previous studies in the literature. Other biologically-plausible confounders (BMI, plasma cobalamin, ever betelnut use, television ownership, and uCr) were considered by examining their bivariate associations with plasma GSH variables and As exposure variables in this dataset; covariates were included in the regression models if they were associated with both exposure and outcome at significance level of 0.2. To reduce extraneous variation in plasma GSH and GSSG, we additionally adjusted these variables for plasma GSH laboratory batch as a categorical variable using the residual method; batch-adjusted plasma GSH and GSSG variables were used in all analyses.

Linear regression models were constructed with plasma GSH variables as predictors and urinary As metabolite variables as the outcomes. Variables with skewed distributions were log-transformed to normalize the distributions of the variables and/or improve the linearity of the relationships between the predictors and outcomes; transformed variables included bAs, wAs, urinary SMI, plasma GSH, plasma GSSG, and plasma GSH/GSSG. Relationships were also examined by using linear models to calculate gender- and wAs-adjusted mean urinary %MMA and %DMA within categories of plasma GSH/GSSG (categorized at tertiles in the overall study sample), stratified by plasma folate status. Differences in the associations between predictor (GSH, GSSG, GSH/GSSG ratio) and outcomes of methylation variables between plasma folate strata were examined and detected by Wald tests. Of the total $n = 378$ participants in the study sample, two participants were excluded for missing data ($n = 1$ participant with missing plasma folate, $n = 1$ participant with missing plasma GSH), leaving $n = 376$ participants in the analysis. All statistical analyses were conducted using SAS (version 9.3; SAS Institute Inc., Cary, NC).

RESULTS

[notdef]Demographic and clinical characteristics of the study participants, overall and by plasma folate status, are shown in **Table 1**. In the overall sample, the average age was 43 y, and there were roughly equal numbers of males and females. Folate-deficient individuals (plasma folate concentrations < 9.0 nmol/L) comprised approximately 29% of the sample. The folate-deficient group had a higher proportion of males, ever cigarette smokers, and betel nut users; higher mean age; and lower mean BMI. The folate-deficient group also had higher %InAs and %MMA and lower %DMA in urine.

Males had higher mean urinary %MMA (16.0% vs. 12.0%, $P < 0.0001$) and lower mean urinary %DMA (66.6% vs. 69.9%, $P < 0.0001$) and plasma GSH/GSSG ratio (1.25 vs. 1.34, $P = 0.07$) than females. As shown in **Table 2**, the plasma GSH/GSSG ratio was negatively correlated with age in both folate groups. In support of previous findings from our group [37], plasma cobalamin was positively correlated with urinary %MMA. Total As exposures in well water, urine, and blood were positively correlated with %InAs and %MMA and negatively correlated with urinary %DMA, consistent with inhibition or saturation of AS3MT by InAs and MMA [16].

In adjusted linear regression models stratified by plasma folate status (**Table 3**), the plasma GSH/GSSG ratio was negatively associated with urinary %MMA (B ± SE, -5.21 ± 1.58, $P = 0.004$) and positively associated with urinary %DMA (B ± SE, 6.53 ± 2.66, $P = 0.04$) in the

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folate-deficient stratum, indicating that a more reduced plasma GSH/GSSG ratio is associated with increased As methylation capacity. No significant associations were observed in the folate-sufficient stratum, and the interactions by folate status were significant for %MMA (P = 0.002) and %DMA (P = 0.01). The plasma GSH/GSSG ratio was not associated with urinary %InAs in adjusted models in either folate stratum (folate-deficient, P = 0.62; folate-sufficient, P = 0.47). The plasma GSH/GSSG ratio was positively associated with urinary SMI in the folate-deficient group (folate-deficient, B ± SE, 0.49 ± 0.14, P = 0.002; folate-sufficient, B ± SE, -0.04 ± 0.07, P = 0.98; P interaction = 0.001). The change in R^2 values for urinary SMI models were 7.7% in the folate-deficient group and 0.0% in the folate-sufficient group; the change in R^2 represents the percentage of the variance in urinary SMI explained by the plasma GSH/GSSG ratio after adjustment for covariates. Additionally, in the folate-deficient group, the plasma GSH/GSSG ratio was negatively associated with total bAs concentrations (B ± SE, -0.39 ± 0.18, P = 0.02). Similar patterns of association were observed when plasma GSH E_h, as calculated by the Nernst equation, was used as the predictor: in adjusted models, a more oxidized plasma GSH E_h was associated with decreased urinary SMI (P = 0.03), increased urinary %MMA (P = 0.03), decreased urinary %DMA (P = 0.17), and increased bAs concentrations (P = 0.08) in the folate-deficient stratum.

To examine whether concentrations of plasma GSH or GSSG were independently associated with As methylation capacity, we built regression models with GSH and GSSG concentrations as predictors of urinary As metabolites, shown in **Table 4**. Since plasma GSH and GSSG were positively correlated with one another (overall sample, Spearman r = 0.29, $P < 0.0001$), we included both variables in the models simultaneously. After adjustment for covariates, in the folate-deficient stratum, plasma GSH was positively associated with urinary SMI (B ± SE, 0.41 ± 0.18, P = 0.04), while plasma GSSG was negatively associated with urinary SMI (B ± SE, -0.53 ± 0.15, P = 0.002). Again, no significant associations were observed in the folate-sufficient stratum.

Least squares mean urinary %MMA and %DMA by plasma GSH/GSSG tertiles, stratified by plasma folate status, are presented in **Figure 2**. In the folate-deficient stratum, the most oxidized plasma GSH/GSSG category (Category 1, ratios of 0.39-1.06) had a 4.4% higher adjusted mean urinary %MMA compared to the least oxidized plasma GSH/GSSG category (Category 3, ratios of 1.42-2.92) (P = 0.0001) (Figure 2A). Category 2 (ratios of 1.07-1.41) had a 2.7% increase in mean urinary %MMA compared to Category 3 (P = 0.01) (Figure 2A). Adjusted mean urinary %MMA did not differ by plasma GSH/GSSG category in the folate-sufficient stratum (Figure 2B). Similarly, Category 1 had a 3.5% lower mean urinary %DMA compared to Category 3 in the folate-deficient (P = 0.07) (Figure 2C), but no differences by category were observed in the folate-sufficient group (Figure 2D).

DISCUSSION

The primary objective of this study was to examine the associations of the plasma GSH/GSSG ratio with indicators of As methylation capacity (urinary %MMA, %DMA, and SMI) and to further examine whether these associations were modified by plasma folate status. In the folate-deficient stratum, we observed that the plasma GSH/GSSG ratio was negatively

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associated with urinary %MMA and positively associated with urinary %DMA and urinary SMI. No significant associations were observed in the folate-sufficient stratum, and the interaction by folate status was statistically significant. Additionally, the plasma GSH/GSSG ratio was negatively associated with total bAs concentrations in the folate-deficient stratum. Our findings suggest that a more reduced plasma GSH redox state is associated with increased As methylation capacity and decreased bAs concentrations, and plasma folate status is a modifier of these associations.

We found that an oxidized plasma GSH redox state was negatively associated with As methylation capacity, with concentrations of plasma GSH and GSSG having independent—and opposite—associations with urinary As metabolite percentages. We speculate that these observations might be explained by *S*-glutathionylation, i.e., direct binding of GSH and GSSG to AS3MT [26]. *In vitro* studies show that biotinylated GSH and GSSG bind to recombinant human AS3MT and that binding of GSH is antagonized by GSSG (David Thomas, unpublished observations, 2013). These interactions are consistent with roles for GSH and GSSG and the GSH/GSSG ratio in regulation of AS3MT activity.

There are several potential explanations why the association between plasma GSH/GSSG and As methylation capacity was only observed in the folate-deficient group. First, it is possible that *S*-glutathionylation of AS3MT, if present, might have a regulatory purpose. For example, GSSG inhibits the Na,K-ATPase enzyme through *S*-glutathionylation of Cys residues in its ATP binding site, but this inhibition does not occur when ATP concentrations are above a certain threshold [38]. The authors speculated that *S*-glutathionylation of Na,K-ATPase is a regulatory mechanism to prevent irreversible loss of ATP under ATP-deficient conditions [38]. It is possible that an analogous mechanism occurs with AS3MT, where exposure to GSSG under folate-deficient conditions increases *S*-glutathionylation of redox-sensitive Cys residues near the SAM binding site, thereby conserving SAM. Interestingly, the AS3MT Cys residues that were previously identified as redox-sensitive, Cys^{156} and Cys^{206}, are located in the SAM binding pocket [39]. Alternatively, mathematical models estimate that hepatic SAM concentrations during folate-sufficient conditions are markedly above the K_m for AS3MT [40], and it is possible that GSH does not measurably modulate the AS3MT methylation rate when SAM concentrations are not limiting. While we cannot definitively establish whether one of these mechanisms explains our observations, it would be of interest to explore these potential mechanisms in experimental models.

It is also possible that our observed associations are explained by differential biliary transport of As metabolite species. For example, efflux of As^{III} and MMA^{III} from hepatocytes to the bile via multidrug resistance protein 2 (Mrp2) requires the formation of As-GSH conjugates [41]. If plasma GSH redox state indeed reflects biliary As excretion, we would expect that plasma GSH redox would be associated with urinary As concentrations. However, we did not observe significant associations between plasma GSH, GSSG, or GSH/GSSG ratio with total urinary As or urinary As metabolite concentrations (InAs, MMA, or DMA) (data not shown). This suggests that the plasma GSH redox state is more strongly associated with the proportion of urinary As metabolites for a given As exposure. The absence of associations between plasma GSH redox and urinary As concentrations also suggests that our observations are less likely to be explained by reverse causality, e.g.,

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inhibition of glutathione reductase (GR) by As metabolites seen at very high As concentrations *in vitro* [42-44] and *in vivo* [45].

We are aware of only one other study that examined the associations between GSH and urinary As metabolites in humans [46]. Xu et al. found that total non-protein sulfhydryls (primarily GSH + GSSG) measured in whole blood was strongly associated with decreased urinary %MMA and increased urinary %DMA in children and adults in Inner Mongolia, China [46]. In our study, we did not observe any significant associations between whole blood GSH or the sum of whole blood GSH and GSSG and urinary As metabolite percentages, either in the overall sample or within the folate strata (data not shown). The reason for this discrepancy is unclear, but it might be related to differing laboratory methodologies used to measure GSH or differences in the study populations. Conversely, numerous studies have examined the associations of genetic variants in the glutathione-*S*-transferase (GST) family of enzymes with urinary As metabolite profiles, although no consistent associations have been observed [47-57]. It is possible that the inconsistent findings might be due to differences in folate nutritional status among the populations included in these studies.

Our study has several limitations. First, we used plasma measurements of GSH, GSSG, and folate as proxies for liver measurements of GSH, GSSG, and SAM. Since the liver is the primary site of As methylation, we would ideally measure GSH, GSSG, and SAM concentrations in hepatic tissue. However, mathematical models of hepatic folate and GSH metabolism indicate that plasma biomarkers might be informative: based on model predictions, plasma folate is strongly related to liver SAM [58], and plasma GSH/GSSG tracks liver GSH/GSSG over a range of steady-state oxidative stress (H_2O_2) concentrations [59]. Second, evidence from Currier et al. suggests that our method for measuring urinary As metabolites, HPLC-ICP-MS, might underestimate the concentrations of the trivalent methylated As metabolites, i.e., MMA^{III} and DMA^{III} [60]. Finally, due to the cross-sectional nature of our study, we are unable to establish the directionality of the relationship between plasma GSH redox status and As methylation capacity.

In conclusion, we found that an oxidized plasma GSH/GSSG redox state was associated with decreased As methylation capacity (increased urinary %MMA, decreased urinary %DMA, and decreased urinary SMI) and increased total bAs among folate-deficient Bangladeshi adults. GSH/GSSG redox might be one of several mechanisms controlling intracellular AS3MT activity and might contribute to the large interindividual variation in As methylation capacity observed in humans.

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Abbreviations

As	arsenic
AS3MT	arsenic(III)-methyltransferase
ATO	arsenic trioxide
bAs	blood arsenic
BMI	body mass index
uCr	urinary creatinine
CV	coefficient of variation
FOX	folate and oxidative stress study
GFAA	graphite furnace atomic absorption
GSH	glutathione
GSSG	glutathione disulfide
ICP-MS	inductively coupled mass spectrometry
SAM	*S*-adenosylmethionine
SMI	secondary methylation index
uAs	urinary arsenic
uDMA	urinary dimethylarsinic acid
uInAs	urinary inorganic arsenic
uMMA	urinary monomethylarsonic acid
wAs	water As

REFERENCES

1. Ahmed MF, et al. Epidemiology. Ensuring safe drinking water in Bangladesh. Science. 2006; 314(5806):1687–8. [PubMed: 17170279]
2. IARC. Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. IARC Monogr Eval Carcinog Risks Hum Suppl. 1987; 7:1–440. [PubMed: 3482203]
3. Navarro Silvera SA, Rohan TE. Trace elements and cancer risk: a review of the epidemiologic evidence. Cancer Causes Control. 2007; 18(1):7–27. [PubMed: 17186419]
4. Rossman TG. Mechanism of arsenic carcinogenesis: an integrated approach. Mutat Res. 2003; 533(1-2):37–65. [PubMed: 14643412]
5. Lin S, et al. A novel S-adenosyl-L-methionine:arsenic(III) methyltransferase from rat liver cytosol. J Biol Chem. 2002; 277(13):10795–803. [PubMed: 11790780]
6. Chiang PK, et al. S-Adenosylmethionine and methylation. FASEB J. 1996; 10(4):471–80. [PubMed: 8647346]
7. Hughes MF, et al. Arsenic (+3 oxidation state) methyltransferase genotype affects steady-state distribution and clearance of arsenic in arsenate-treated mice. Toxicol Appl Pharmacol. 2010; 249(3):217–23. [PubMed: 20887743]
8. Gebel TW. Arsenic methylation is a process of detoxification through accelerated excretion. International Journal of Hygiene and Environmental Health. 2002; 205(6):505–508. [PubMed: 12455273]

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9. Styblo M, et al. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Arch Toxicol. 2000; 74(6):289–99. [PubMed: 11005674]
10. Petrick JS, et al. Monomethylarsonous acid (MMA(III)) and arsenite: LD(50) in hamsters and in vitro inhibition of pyruvate dehydrogenase. Chem Res Toxicol. 2001; 14(6):651–6. [PubMed: 11409934]
11. Vahter M, Concha G. Role of metabolism in arsenic toxicity. Pharmacol Toxicol. 2001; 89(1):1–5. [PubMed: 11484904]
12. Smith AH, Steinmaus CM. Health effects of arsenic and chromium in drinking water: recent human findings. Annu Rev Public Health. 2009; 30:107–22. [PubMed: 19012537]
13. Tseng CH. Arsenic methylation, urinary arsenic metabolites and human diseases: current perspective. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2007; 25(1):1–22. [PubMed: 17365340]
14. Thomas DJ. Unraveling arsenic--glutathione connections. Toxicol Sci. 2009; 107(2):309–11. [PubMed: 19074764]
15. Jones DP. Radical-free biology of oxidative stress. American Journal of Physiology - Cell Physiology. 2008; 295(4):C849–C868. [PubMed: 18684987]
16. Song X, et al. New insights into the mechanism of arsenite methylation with the recombinant human arsenic (+3) methyltransferase (hAS3MT). Biochimie. 2010; 92(10):1397–406. [PubMed: 20621156]
17. Waters SB, et al. Glutathione modulates recombinant rat arsenic (+3 oxidation state) methyltransferase-catalyzed formation of trimethylarsine oxide and trimethylarsine. Chem Res Toxicol. 2004; 17(12):1621–9. [PubMed: 15606138]
18. Waters SB, et al. Endogenous reductants support the catalytic function of recombinant rat cyt19, an arsenic methyltransferase. Chem Res Toxicol. 2004; 17(3):404–9. [PubMed: 15025511]
19. Thomas DJ, et al. Arsenic (+3 oxidation state) methyltransferase and the methylation of arsenicals. Exp Biol Med (Maywood). 2007; 232(1):3–13. [PubMed: 17202581]
20. Ding L, et al. Methylation of arsenic by recombinant human wild-type arsenic (+3 oxidation state) methyltransferase and its methionine 287 threonine (M287T) polymorph: Role of glutathione. Toxicol Appl Pharmacol. 2012; 264(1):121–30. [PubMed: 22868225]
21. Challenger F. Biological methylation. Adv Enzymol Relat Subj Biochem. 1951; 12:429–91. [PubMed: 14885024]
22. Challenger F. Biological methylation. Chemical Reviews. 1945; 36(3):315–361.
23. Hayakawa T, et al. A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19. Arch Toxicol. 2005; 79(4):183–91. [PubMed: 15526190]
24. Wang S, et al. Rapid equilibrium kinetic analysis of arsenite methylation catalyzed by recombinant human arsenic (+3 oxidation state) methyltransferase (hAS3MT). J Biol Chem. 2012; 287(46): 38790–9. [PubMed: 22955273]
25. Fomenko DE, et al. High-Throughput Identification of Catalytic Redox-Active Cysteine Residues. Science. 2007; 315(5810):387–389. [PubMed: 17234949]
26. Dalle-Donne I, et al. S-glutathionylation in protein redox regulation. Free Radic Biol Med. 2007; 43(6):883–98. [PubMed: 17697933]
27. Kombu RS, et al. Dynamics of glutathione and ophthalmate traced with 2H-enriched body water in rats and humans. American Journal of Physiology - Endocrinology And Metabolism. 2009; 297(1):E260–E269. [PubMed: 19401458]
28. Hall MN, et al. Chronic Arsenic Exposure and Blood Glutathione and Glutathione Disulfide Concentrations in Bangladeshi Adults. Environ Health Perspect. 2013
29. Cheng Z, et al. Rapid multi-element analysis of groundwater by high-resolution inductively coupled plasma mass spectrometry. Anal Bioanal Chem. 2004; 379(3):512–8. [PubMed: 15098084]
30. Van Geen A, et al. Reliability of a commercial kit to test groundwater for arsenic in Bangladesh. Environ Sci Technol. 2005; 39(1):299–303. [PubMed: 15667109]

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31. van Geen A, et al. Monitoring 51 community wells in Araihazar, Bangladesh, for up to 5 years: implications for arsenic mitigation. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2007; 42(12):1729–40. [PubMed: 17952774]
32. Vela NP, Heitkemper DT, Stewart KR. Arsenic extraction and speciation in carrots using accelerated solvent extraction, liquid chromatography and plasma mass spectrometry. Analyst. 2001; 126(7):1011–7. [PubMed: 11478628]
33. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scand J Clin Lab Invest. 1965; 17(4):381–7. [PubMed: 5838275]
34. Hall M, et al. Blood arsenic as a biomarker of arsenic exposure: results from a prospective study. Toxicology. 2006; 225(2-3):225–33. [PubMed: 16860454]
35. Jones DP, et al. Glutathione measurement in human plasma. Evaluation of sample collection, storage and derivatization conditions for analysis of dansyl derivatives by HPLC. Clin Chim Acta. 1998; 275(2):175–84. [PubMed: 9721075]
36. Jones DP, et al. Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses. Free Radic Biol Med. 2002; 33(9):1290–300. [PubMed: 12398937]
37. Hall MN, et al. Influence of cobalamin on arsenic metabolism in Bangladesh. Environ Health Perspect. 2009; 117(11):1724–9. [PubMed: 20049124]
38. Petrushanko IY, et al. S-glutathionylation of the Na,K-ATPase catalytic alpha subunit is a determinant of the enzyme redox sensitivity. J Biol Chem. 2012; 287(38):32195–205. [PubMed: 22798075]
39. Li X, et al. Functional evaluation of Asp76, 84, 102 and 150 in human arsenic(III) methyltransferase (hAS3MT) interacting with S-adenosylmethionine. FEBS Letters. (0)
40. Lawley S, et al. Mathematical model insights into arsenic detoxification. Theoretical Biology and Medical Modelling. 2011; 8(1):31. [PubMed: 21871107]
41. Leslie EM. Arsenic–glutathione conjugate transport by the human multidrug resistance proteins (MRPs/ABCCs). Journal of Inorganic Biochemistry. 2012; 108(0):141–149. [PubMed: 22197475]
42. Styblo M, Thomas DJ. In vitro inhibition of glutathione reductase by arsenotriglutathione. Biochem Pharmacol. 1995; 49(7):971–7. [PubMed: 7741769]
43. Styblo M, et al. Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. Chem Res Toxicol. 1997; 10(1):27–33. [PubMed: 9074799]
44. Chouchane S, Snow ET. In vitro effect of arsenical compounds on glutathione-related enzymes. Chem Res Toxicol. 2001; 14(5):517–22. [PubMed: 11368549]
45. Rodríguez VM, et al. Glutathione Reductase Inhibition and Methylated Arsenic Distribution in Cd1 Mice Brain and Liver. Toxicological Sciences. 2005; 84(1):157–166. [PubMed: 15601678]
46. Xu Y, et al. Association of oxidative stress with arsenic methylation in chronic arsenic-exposed children and adults. Toxicol Appl Pharmacol. 2008; 232(1):142–9. [PubMed: 18640141]
47. Chiou HY, et al. Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutat Res. 1997; 386(3):197–207. [PubMed: 9219558]
48. Caceres DD, et al. Polymorphism of glutathione S-transferase (GST) variants and its effect on distribution of urinary arsenic species in people exposed to low inorganic arsenic in tap water: an exploratory study. Arch Environ Occup Health. 2010; 65(3):140–7. [PubMed: 20705574]
49. Schlawicke Engstrom K, et al. Genetic polymorphisms influencing arsenic metabolism: evidence from Argentina. Environ Health Perspect. 2007; 115(4):599–605. [PubMed: 17450230]
50. Lindberg AL, et al. Metabolism of low-dose inorganic arsenic in a central European population: influence of sex and genetic polymorphisms. Environ Health Perspect. 2007; 115(7):1081–6. [PubMed: 17637926]
51. Paiva L, et al. Association between GSTO2 polymorphism and the urinary arsenic profile in copper industry workers. Environ Res. 2010; 110(5):463–8. [PubMed: 20045512]
52. Steinmaus C, et al. Genetic polymorphisms in MTHFR 677 and 1298, GSTM1 and T1, and metabolism of arsenic. J Toxicol Environ Health A. 2007; 70(2):159–70. [PubMed: 17365577]

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53. Chung CJ, et al. Polymorphisms in one-carbon metabolism pathway genes, urinary arsenic profile, and urothelial carcinoma. Cancer Causes Control. 2010; 21(10):1605–13. [PubMed: 20532609]
54. Agusa T, et al. Individual variations in arsenic metabolism in Vietnamese: the association with arsenic exposure and GSTP1 genetic polymorphism. Metallomics. 2012; 4(1):91–100. [PubMed: 22028001]
55. Hwang YH, et al. Genetic polymorphism of As3MT and delayed urinary DMA excretion after organic arsenic intake from oyster ingestion. J Environ Monit. 2010; 12(6):1247–54. [PubMed: 20532380]
56. Ahsan H, et al. Arsenic Metabolism, Genetic Susceptibility, and Risk of Premalignant Skin Lesions in Bangladesh. Cancer Epidemiology Biomarkers & Prevention. 2007; 16(6):1270–1278.
57. Marnell LL, et al. Polymorphisms in the human monomethylarsonic acid (MMA V) reductase/hGSTO1 gene and changes in urinary arsenic profiles. Chem Res Toxicol. 2003; 16(12):1507–13. [PubMed: 14680363]
58. Duncan TM, Reed MC, Nijhout HF. The relationship between intracellular and plasma levels of folate and metabolites in the methionine cycle: a model. Mol Nutr Food Res. 2013; 57(4):628–36. [PubMed: 23143835]
59. Reed MC, et al. A mathematical model of glutathione metabolism. Theor Biol Med Model. 2008; 5:8. [PubMed: 18442411]
60. Currier J, et al. Comparative oxidation state specific analysis of arsenic species by high-performance liquid chromatography-inductively coupled plasma-mass spectrometry and hydride generation-cryotrapping-atomic absorption spectrometry. J Anal At Spectrom. 2013; 28(6):843–852. [PubMed: 23687401]

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Highlight

- Arsenic (As) is methylated to MMA and DMA in reductant-dependent reactions.
- We examined interactions of GSH redox and folate status on As methylation capacity.
- Plasma GSH, GSSG and folate and urinary As metabolites were measured in 376 adults.
- Low GSH/GSSG was associated with higher %MMA and lower %DMA in folate deficiency.
- Oxidized GSH redox and low folate may interact to reduce As methylation capacity.

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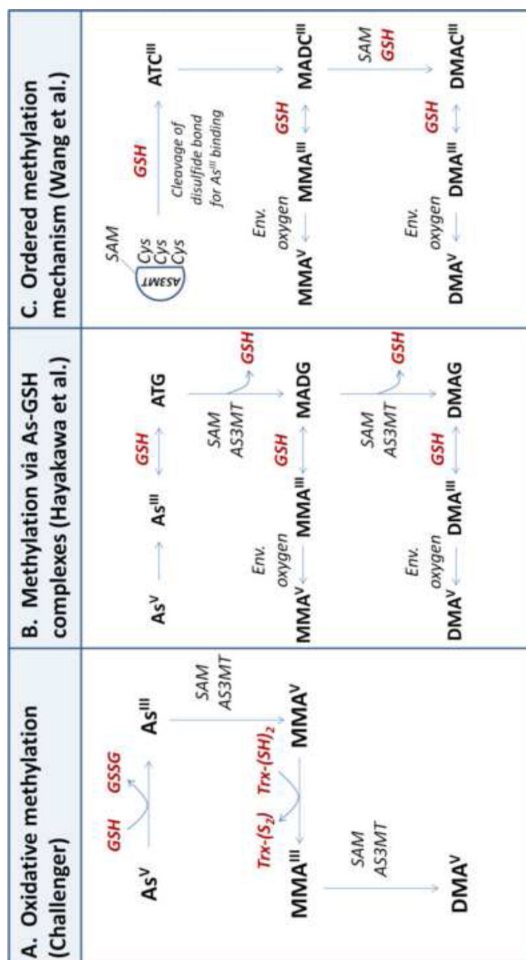

Figure 1. Involvment of glutathione in arsenic methylation mechanisms

Inorganic arsenic (InAs) is metabolized in the liver via a series of methylation reactions, resulting in the formation of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Glutathione (GSH), while not absolutely required for As methylation, can serve as the reductant necessary for AS3MT activity. Three distinct AS3MT-catalyzed methylation pathways have been proposed in the literature (A-C):

A. *Oxidative methylation*. In this pathway first proposed by Challenger [21, 22], AS3MT catalyzes the oxidative methylation of arsenite (As^{III}) and methylarsonous acid (MMA^{III}) to methylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}), respectively. A reductant (such as GSH) is required for the reduction of the pentavalent arsenicals (As^{V} and MMA^{V}) to their trivalent counterparts (As^{III} and MMA^{III}).

B. *Successive methylation via As-GSH complexes*. Hayakawa et al. [23] proposes a pathway in which As-GSH complexes are substrates for AS3MT. Here, As^{III} nonenzymatically complexes with GSH to generate arsenic triglutathione (ATG), which is methylated by AS3MT to form monomethylarsonic diglutathione (MADG). MADG can be further methylated to dimethylarsinic glutathione (DMAG). As-GSH complexes (ATG, MADG, and DMAG) are in equilibrium with trivalent arsenicals (As^{III}, MMA^{III}, and DMA^{III}) based on the concentration of GSH. The trivalent methylated arsenicals (MMA^{III} and DMA^{III}) can be oxidized to pentavalent arsenicals (MMA^{V} and DMA^{V}) by environmental oxygen.

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C. *Ordered methylation mechanism.* Wang et al. [24] propose a model in which a reductant (thiol or nonthiol) changes the conformation of AS3MT to influence enzyme activity. In this scheme, SAM binds to AS3MT; then, a reductant (such as GSH) cleaves a disulfide bond in the enzyme, which exposes active-site cysteine (Cys) residues for As^{III} binding. As^{III} binds the Cys residues, forming arsenic tricysteine (ATC^{III}), and the methyl group from SAM is transferred to ATC^{III} on the AS3MT enzyme, resulting in monomethylarsonic dicysteine ($MADC^{III}$). $MADC^{III}$ can remain bound to AS3MT and be further methylated to dimethylarsinic cysteine ($DAMC^{III}$). $MADC^{III}$ and $DAMC^{III}$ can dissociate from AS3MT, forming MMA^{III} and DMA^{III}, respectively. Trivalent species are then oxidized to pentavalent arsenicals by environmental oxygen.

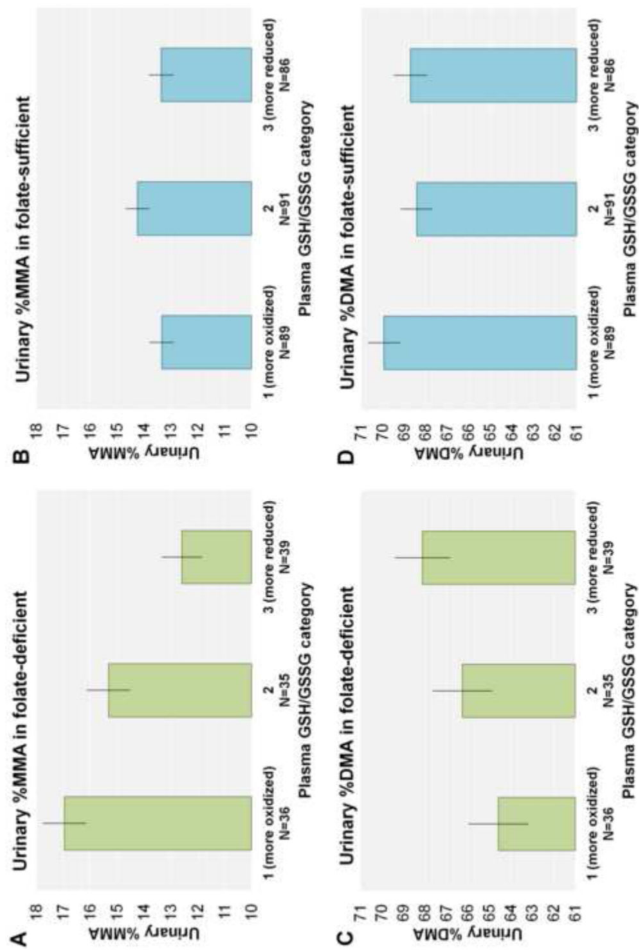

Figure 2. Adjusted mean urinary %MMA and %DMA by plasma GSH/GSSG tertile, stratified by plasma folate status

Bars represent mean ± SE urinary %MMA and %DMA for each plasma GSH/GSSG tertile, adjusted for gender and well water As.

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Table 1

Descriptive characteristics for study sample by plasma folate status.

Baseline variables	**Total sample *N*=376**	**Folate deficient (<9.0 nmol/L) *N*=110**	**Folate sufficient (9.0 nmol/L) *N*=266**	**Group difference *P***
Age (yrs)	43.1±8.03[a] (30 to 63)	44.4±8.0 (30 to 62)	42.6±8.3 (31 to 63)	0.02
Male	182 (48.4)[b]	70 (64.6)	112 (42.1)	<0.0001
BMI (kg/m^2)	20.4±3.5 (13.8 to 35.3)[c]	19.7±3.0 (13.8 to 31.7)	20.7±3.6 (14.5 to 35.3)[e]	0.02
Ever cigarette smoking	136 (36.2)	59 (53.6)	77 (29.0)	<0.0001
Ever betel nut use	160 (42.6)	59 (53.6)	101 (38.0)	0.003
Television ownership	219 (58.2)	57 (51.8)	162 (60.9)	0.07
Plasma folate (nmol/L)	12.9±7.2 (2.4 to 60.6)	7.1±1.4 (2.4 to 8.9)	15.3±7.2 (9.1 to 60.6)	<0.0001
Plasma cobalamin (μM)	204±113 (44 to 1183)[d]	191±93 (59 to 557)	209±119 (44 to 1183)[f]	0.20
Plasma GSH (μmol/L)	2.62±0.71 (1.00 to 5.52)	2.61±0.72 (1.22–4.55)	2.62±0.71 (1.00 to 5.52)	0.94
Plasma GSSG (μmol/L)	2.14±0.60 (0.81 to 4.66)	2.07±0.65 (0.92–4.66)	2.16±0.58 (0.81 to 4.04)	0.06
Plasma GSH/GSSG	1.29±0.44 (0.39 to 3.02)	1.34±0.44 (0.59 to 2.67)	1.28±0.45 (0.39 to 3.02)	0.21
Plasma GSH E_h (mV)	–98.7±7.3 (–118.0 to –73.4)	–98.6±6.8 (–112.6 to –83.6)	–98.7±7.5 (–118.0 to –73.4)	0.92
Water As (μg/L)	138±124 (0.4 to 700)	164±143 (0.4 to 700)	128±114 (0.4 to 447)	0.04
Blood As, total (μg/L)	13.4±9.8 (1.2 to 57.0)	15.4±10.9 (1.3 to 51.3)	12.5±9.1 (1.2 to 57.0)	0.03
Urinary As, total (μg/L)	202±226 (3 to 1990)	209±207 (7 to 992)	119±223 (3 to 1990)	0.43
Urinary Cr (mg/dL)	53±44 (4 to 224)	53±42 (4 to 212)	55±44 (6 to 224)	0.83
Urinary As/Cr (μg/g Cr)	417±329 (16 to 1832)	464±361 (16 to 1743)	397±312 (18 to 1832)	0.14
Urinary %InAs	17.7±5.5 (6.7 to 51.8)	18.6±5.9 (8.3 to 42.9)	17.4±5.3 (6.7 to 51.8)	0.05
Urinary %MMA	13.9±5.0 (3.6 to 30.0)	15.3±5.5 (3.6 to 30.0)	13.4±4.7 (4.2 to 28.5)	0.002
Urinary %DMA	68.3±7.9 (38.3 to 88.0)	66.1±8.5 (39.5 to 88.0)	69.3±7.4 (38.3 to 85.7)	0.004

[a]Mean±SD (range) (all such values)

[b]N(%) (all such values)

[c]N=375

[d]N=370

[e]N=265

[f]N=260.

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Table 2

Spearman correlations of continuous sample characteristics with plasma glutathione variables and urinary As metabolite percentages.

Total sample, N=376				
	Plasma GSH/GSSG	**Urinary %InAs**	**Urinary %MMA**	**Urinary %DMA**
Age	–0.23***	–0.17**	0.15**	0.01
BMI[a]	–0.12*	–0.05	–0.18**	0.14**
Plasma cobalamin[b]	–0.15**	–0.08	0.13*	–0.04
Water As	0.09	0.21***	0.21***	–0.26***
Urinary As/Cr	0.05	0.25**	0.21***	–0.27***
Blood As	0.03	0.25***	0.30***	–0.35***
0.5				
Folate deficient, N=110				
Age	–0.29***	–0.24**	0.23**	–0.01
BMI	–0.19*	–0.08	–0.22**	0.20**
Plasma cobalamin	–0.17	0.05	0.13	–0.10
Water As	0.18	0.21*	0.29***	–0.27***
Urinary As/Cr	0.13	0.27**	0.23*	–0.30**
Blood As	0.05	0.29**	0.36**	–0.38***
0.5				
Folate sufficient, N=266				
Age	–0.22***	–0.17**	0.09	0.05
BMI[c]	–0.09	–0.03	–0.14*	0.10
Plasma cobalamin[d]	–0.14*	–0.13*	0.14*	–0.02
Water As	0.03	0.20**	0.14**	–0.22**
Urinary As/Cr	0.01	0.18**	0.18**	–0.23**
Blood As	0.01	0.22**	0.25***	–0.31***

[a] N=375

[b] N=370

[c] N=265

[d] N=260

* p<0.05

** p<0.01

*** $p<0.0001$.

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Table 3

Regression coefficients for associations between log-transformed plasma GSH/GSSG ratio and arsenic methylation capacity indicators, stratified by plasma folate status.

Outcome	Model	Total sample N=376	Folate deficient N=110			Folate sufficient N=266			*P* interaction[b]
		B±SE	B+SE	R^2 (%)	in R^2 (%)	B±SE	R^2 (%)	in R^2 (%)	
Urinary %MMA	Adj. for gender, age, and wAs[c]	–1.03±0.70	–4.60±1.51[**]	29.6	6.4	–0.18±0.77	19.2	0.0	0.001
	Extended model[a]	–0.81±0.71	–5.21±1.58[**]	36.3	7.1	0.17±0.78	24.8	0.0	0.002
Urinary %DMA	Adj. for gender, age, and wAs[c]	0.09±1.16	4.93±2.56[†]	15.2	2.9	–0.92±1.29	12.0	0.2	0.04
	Extended model[a]	0.28±1.20	6.53±2.66[*]	23.4	4.5	–0.89±1.33	13.7	0.1	0.01
Urinary SMI[c]	Adj. for gender, age, and wAs[c]	0.07±0.07	0.41±0.14[**]	29.9	6.3	–0.01±0.07	16.9	0.0	0.007
	Extended model[a]	0.06±0.07	0.49±0.14[**]	37.1	7.7	–0.04±0.07	23.0	0.0	0.001
Blood As[c]	Adj. for gender, age, and wAs[c]	–0.04±0.08	–0.26±0.17	61.6	0.8	0.02±0.09	62.4	0.0	0.15
	Extended model[a]	–0.10±0.08	–0.39±0.18[*]	63.5	1.6	–0.01±0.09	64.1	0.0	0.06

[a]Adjusted for gender, age, log-transformed water As, cigarette smoking (ever/never), betelnut chewing (ever/never), television ownership, plasma cobalamin, and BMI; N=110 in folate deficient, N=259 in folate sufficient

[b]p-value from Wald test for group difference in the regression coefficient.

[c]Log-transformed.

[†]$p<0.10$

[*]$p<0.05$

[**]$p<0.01$.

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Table 4

Regression coefficients for associations between log-transformed plasma GSH and plasma GSSG concentrations and arsenic methylation capacity indicators, stratified by plasma folate status.

Outcome	Predictor	Total sample N=376	Folate-deficient N=110	Folate-sufficent N=266	*P* interaction[b]
		B±SE	B±SE	B±SE	
Urinary %MMA	Plasma GSH[a]	–0.02±0.88	–4.75±2.00[*]	1.16±0.98	0.008
	Plasma GSSG[a]	1.59±0.87[†]	5.46±1.72[**]	0.93±1.02	0.0003
Urinary %DMA	Plasma GSH[a]	–0.60±1.50	4.93±3.37	–1.24±1.68	0.10
	Plasma GSSG[a]	–1.13±1.48	–7.40±2.90[*]	0.50±1.76	0.02
Urinary SMI[c]	Plasma GSH[a]	–0.04±0.08	0.41±0.18[*]	–0.14±0.09	0.006
	Plasma GSSG[a]	–0.15±0.08[†]	–0.53±0.15[**]	–0.07±0.10	0.01
Blood As[c]	Plasma GSH[a]	–0.10±0.10	–0.34±0.22	–0.05±0.12	0.25
	Plasma GSSG[a]	0.09±0.10	0.41±0.19[*]	–0.03±0.12	0.05

[a]Adjusted for gender, age, log-transformed water As, cigarette smoking (ever/never), betelnut chewing (ever/never), television ownership, plasma cobalamin, BMI, and other plasma variable (GSH or GSSG); N=110 in folate deficient, N=259 in folate sufficient.

[b]p-value from Wald test for group difference in the regression coefficient.

[c]Log-transformed.

[†]$p<0.10$

[*]$p<0.05$

[**]$p<0.01$.