Influence of Cobalamin on Arsenic Metabolism in Bangladesh

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**Background:** Arsenic is a carcinogen to which 35 million people in Bangladesh are chronically exposed. The enzymatic transfer of methyl groups to inorganic As (iAs) generates monomethylarsonic (MMA) and dimethylarsinic acids (DMA) and facilitates urinary As (uAs) elimination. This process is dependent on one-carbon metabolism, a pathway in which folate and cobalamin have essential roles in the recruitment and transfer of methyl groups. Although DMAV is the least toxic metabolite, increasing evidence suggests that MMAIII may be the most cytotoxic and genotoxic As intermediary metabolite.

**Objective:** We examined the associations between plasma cobalamin and uAs metabolites.

**Methods:** We conducted a cross-sectional study of 778 Bangladesh adults in which we oversampled cobalamin-deficient participants. Participants provided blood samples for the measurement of plasma cobalamin and urine specimens for As measurements.

**Results:** Cobalamin was inversely associated with the proportion of total uAs excreted as iAs (%iAs) (unstandardized regression coefficient (b) = –0.10; 95% confidence interval (CI), –0.17 to –0.02; p = 0.01) and positively associated with %MMA (b = 0.12; 95% CI, 0.05 to 0.20; p = 0.001). Both of these associations were stronger among folate-sufficient participants (%iAs: b = –0.17; 95% CI, –0.30 to –0.03; p = 0.02; %MMA: b = 0.20; 95% CI, 0.11 to 0.30; p < 0.0001), and the differences by folate status were statistically significant.

**Conclusions:** In this group of Bangladesh adults, cobalamin appeared to facilitate the first As methylation step among folate-sufficient individuals. Given the toxicity of MMAIII, our findings suggest that in contrast to folate, cobalamin may not favorably influence As metabolism.

**Keywords:** arsenic, Bangladesh, cobalamin, creatinine, dimethylarsinic acid, folate, homocysteine, monomethylarsonic acid, one-carbon metabolism. Environ Health Perspect 117:1724–1729 (2009). doi:10.1289/ehp.0900734 available via http://dx.doi.org/ [Online 31 July 2009]

Ingestion of arsenic through contaminated drinking water is a major health concern in Bangladesh, where approximately 35 million people are chronically exposed (Kinniburgh and Smedley 2001). This exposure has been linked to increased risks of cancers of the skin, bladder, liver, and lung [California Environmental Protection Agency (EPA) 2004; Navarro et al. 2007], as well as ischemic heart disease and neurologic impairments (Navarro et al. 2007).

Metabolism of As occurs through a series of reduction and methylation reactions (Figure 1). The methylation steps are catalyzed by arsenic methyltransferase (AS3MT), with S-adenosylmethionine (SAM) serving as the methyl donor (Lin et al. 2002; Marafante and Vahter 1984). Inorganic arsenite is first methylated to monomethylarsonic acid (MMAV). Upon reduction to MMAIII, it then undergoes a second methylation to dimethylarsinic acid (DMAI). Methylation has generally been considered a detoxification pathway that generates the least toxic organic mammmalian arsical metabolite, DMAV, and facilitates urinary excretion (Vahter and Marafante 1987). However, increasing evidence suggests that MMAIII is a highly cytotoxic and genotoxic intermediate (Styblo et al. 2002; Wang et al. 2007).

Folate, cobalamin (vitamin B12), and vitamin B6 are required for the recruitment and transfer of methyl groups in one-carbon metabolism. Other nutrients, including protein, betaine, and choline, also contribute to the availability of methyl groups ultimately used in SAM biosynthesis. Arsenic retention in tissues is increased with dietary methyl donor deficiency (Vahter and Marafante 1987), and urinary As (uAs) excretion is decreased in folate deficiency (Spiegelstein et al. 2003). Our previous work has shown that individual variation in the ability to methylate As is associated with folate status (Gamble et al. 2005b; 2006; 2007). However, few investigations of the association between cobalamin and As metabolism have been conducted, despite its critical role as an essential cofactor for methionine synthase, which catalyzes the remethylation of homocysteine to methionine, a critical step in SAM biosynthesis. Zakharyan and Aposhian (1999) reported that in vitro incubation of methylcobalamin (CH3B12) with arsenite in the presence of a reducing agent can produce MMA and trace amounts of DMA nonenzymatically. We observed no association between plasma cobalamin and uAs metabolites in our previous cross-sectional study of 300 adults (Gamble et al. 2005b).

Because the primary aim of that study was to examine the influence of folate on As methylation, we excluded cobalamin-deficient participants, thereby restricting the range of plasma cobalamin values.

To examine the influence of a full range of plasma cobalamin concentrations on As metabolism, we conducted a cross-sectional study in which we oversampled cobalamin-deficient participants (plasma cobalamin < 185 pmol/L). We also set out to replicate the in vitro work of Zakharyan and Aposhian (1999) in a slightly modified system, as this finding would contribute to our understanding of the role that cobalamin might play in influencing the metabolism of As.

**Materials and Methods**

**Study participants, procedure, and ethics.** Using biologic samples from our original cross-sectional study of a random sample of 1,650 adults [the Nutritional Influences on Arsenic Toxicity study (Gamble et al. 2005a)], we analyzed uAs metabolites for all of the cobalamin-deficient participants (n = 412 with plasma cobalamin < 185 pmol/L). For efficiency, we combined these individuals with an additional 366 nondeficient participants who already had uAs metabolite data, to incorporate a broad range of cobalamin nutritional status. This process yielded a total of 778 participants. All participants had provided blood and urine samples, and demographic information was obtained from a baseline survey. All study subjects gave informed oral consent to Bangladeshi field staff physicians before participating in the study. The research protocol was approved by the Institutional Review Board of Columbia University and the Bangladesh Medical Research Council.

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Collection of biospecimens. Blood samples were collected by venipuncture from participants who had been sitting for 10–15 min. The samples were collected into EDTA-containing tubes that were immediately placed in IsoRack cool packs (Brinkmann Instruments, Westbury, NY, USA). Samples were transported by hand (in coolers containing additional ice packs) to our field clinic in Araizar within 4 hr of collection. The samples were then centrifuged at 4°C to separate plasma and cells. Plasma was stored at –80°C and shipped frozen on dry ice to Columbia University for analysis. Urine samples were collected in 50-mL acid-washed tubes, frozen at –20°C, and shipped on dry ice.

Measurement of plasma nutrients. Plasma cobalamin and folate were analyzed by radioimmunoassay (QuantaPhase II; Bio-Rad Laboratories, Richmond, CA, USA) as previously described (Gamble et al. 2005a; Pfeiffer et al. 2005). The within-day coefficient of variation (CV) was 3% for folate and 4% for cobalamin. The between-day CV was 11% for folate and 8% for cobalamin. We measured plasma total homocysteine (tHcy) using high-performance liquid chromatography (HPLC) with fluorescence detection (Pfeiffer et al. 1999) as described previously (Gamble et al. 2005a).

Well water As. We used graphite furnace atomic absorption (GFAA), with a detection limit of 5 µg/L, to measure total As in well-water samples. Samples found to have a concentration < 5 µg/L were reanalyzed by inductively coupled plasma-mass spectrometry (ICP-MS), for which the detection limit was 0.1 µg/L (Cheng et al. 2004).

Total uAs and creatinine. Total uAs was measured in the Columbia University Trace Metals Core Laboratory by GFAA spectrometry using the Analyst 600 graphite furnace system (PerkinElmer, Shelton, CT, USA), according to the method of Nixon et al. (1991). Our laboratory participates in a quality control program organized by Philippe Weber at the Quebec Toxicology Center in Quebec, Canada. Intraclass correlation coefficients for every combination of pH and molar ratio. The reaction mixture was incubated for 1.5, 3, 6, 9, 12, and 30 hr at 37°C. At each time point we aliquoted 50 µL of the mixture, diluted it with mobile phase (10 mM ammonium phosphate plus 10 mM ammonium nitrate), injected it onto the HPLC column, and detected concentrations of MMA, DMA, AsIII, and AsV by ICP-MS with dynamic reaction cell technology (ICP-MS-DRC). Calibration standards of a mixture of As metabolites were similarly processed. ICP-MS-DRC coupled to HPLC separates and detects 7 As metabolites chromatographically separated by ion exchange with the use of a PRP-X100 column (Hamilton Co, Reno, NV, USA).

Statistical analysis. Plasma nutrients and uAs metabolites were not normally distributed. We therefore used nonparametric tests where appropriate and natural log transformations that may either create approximately normal distributions for dependent variables in linear regression analysis, improve linearity in the relationship between an independent and dependent variable, or reduce the impact of extreme values of an independent variable in fitting linear models. We calculated descriptive statistics separately by sex for general characteristics as well as for plasma nutrient and uAs metabolite concentrations. We tested for sex differences using nonparametric tests.

Figure 1. Arsenate is reduced to arsenite in a reaction thought to be dependent on GSH or other endogenous reductants. Arsenite then undergoes an oxidative methylation, with SAM as the methyl donor, forming MMAV and SAH. MMAV is reduced to MMAIII and then undergoes a subsequent oxidative methylation step to produce DMAV and SAH.
differences using the Wilcoxon rank-sum test for continuous variables and the chi-square test for categorical variables. The correlations between plasma cobalamin and covariates and between cobalamin and the percentage of As metabolites in urine were examined using Spearman’s correlation coefficients. We used linear regression models to examine associations between plasma cobalamin and the outcome variables %iAs, %MMA, and %DMA in urine. Potential confounders considered for inclusion in the regression models were age, sex, urinary creatinine, body mass index (BMI), and total uAs. We used a Wald test to detect differences in the covariate-adjusted associations between cobalamin and uAs metabolites by sex, folate status, or water As status. We ran separate linear regression models to examine the covariate-adjusted associations between plasma cobalamin and each of the uAs metabolites and to examine the differences in these associations by sex, folate status, or water As status. Analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-sided with a significance level of 0.05. The As metabolite concentrations detected in the nonenzymatic laboratory assay experiments were expressed as a percentage of total detected metabolites.

Results

Cross-sectional study of cobalamin and uAs metabolites. The general characteristics and plasma nutrient concentrations of the study participants by sex are shown in Table 1. The men in this sample were significantly older than the women and had a lower mean BMI. Nearly 60% of men were current cigarette smokers compared with 3% of women. Both women (30.6%) and men (40.9%) had a high prevalence of current betel nut use. Water As concentrations of the wells that served as the primary source of drinking water were above the Bangladeshi standard (50 µg/L) for 60% of women and 58% of men. The water As concentrations were above the World Health Organization standard (10 µg/L) for 81% of women and 83% of men.

Because we oversampled cobalamin-deficient participants, a large proportion of the sample had cobalamin deficiency: 57.6% of women and 46.8% of men had < 185 pmol/L. We used cutoff values of < 9.0 nmol/L for folate deficiency (Christenson et al. 1985), and ≥ 10.4 for women and ≥ 11.4 µmol/L for men for hyperhomocysteinemia [National Health and Nutrition Examination Survey III (Selhub et al. 1999)]; folate deficiency and hyperhomocysteinemia were very common, particularly in men (Table 1). We observed an inverse correlation between plasma cobalamin and tHcy concentrations (females: r = −0.26, p < 0.0001; males: r = −0.47, p < 0.0001).

Mean water As concentrations did not differ significantly by sex (Table 2). As expected, mean urinary creatinine concentrations were higher among males than among females, although this difference did not achieve statistical significance in this sample (Table 2). Mean concentrations of uAs were similar for males and females; however, uAs per gram creatinine (uAs/gCr) was significantly higher among females than among males. Women had significantly lower percentages of As in urine present as MMA (%MMA) and higher as DMA

Table 1. General characteristics of the study sample.

| Characteristic | Female (n = 479) | Male (n = 299) | p-Value
|----------------|-----------------|----------------|-----------------|
| Age (years)    | 33.7 ± 8.8      | 42.9 ± 10.2    | <0.0001         |
| Height (cm)    | 149.7 ± 5.2     | 161.6 ± 5.5    | <0.0001         |
| Weight (kg)    | 45.2 ± 7.7      | 50.7 ± 6.3     | <0.0001         |
| BMI (kg/m²)    | 20.2 ± 3.1      | 19.4 ± 2.8     | 0.0001          |
| BMI < 18.5 (%) | 31.6            | 45.6           | <0.0001         |
| Male (%)       | 283.0 (262.7–303.2) | 149.6 (133.0–165.0) | <0.0001         |
| Education (years) | 3.1 ± 3.5   | 3.8 ± 3.9      | 0.02            |
| Type of housing (%) | 7.7        | 7.4            | 0.49            |
| Thatched       | 7.7            | 7.4            | 0.49            |
| Corrugated     | 77.7           | 57.9           | 0.46            |
| Water As > 50 µg/L (%) | 60.9    | 81.4           | 0.51            |
| Plasma cobalamin (pmol/L) | 217.3 ± 114.6 | 217.3 ± 117.2 | 0.0006          |
| Plasma cobalamin < 151 pmol/L (%) | 26.5 | 19.4 | 0.02 |
| Plasma cobalamin < 185 pmol/L (%) | 57.6 | 46.8 | 0.003 |
| Plasma folate (µg/L) | 132.9 ± 93 | 9.4 ± 7.1 | <0.0001 |
| Plasma folate < 5.0 µmol/L (%) | 42.0 | 69.2 | <0.0001 |
| Plasma tHcy (µmol/L) | 9.7 ± 5.0 | 16.5 ± 11.8 | <0.0001 |
| Hyperhomocysteinemia (%) | 29.2 | 65.1 | <0.0001 |

*p-Value for test of difference by sex, based on Wilcoxon’s rank-sum test for continuous variables and chi-square or Fisher’s exact test for categorical variables. *Based on reference values from Christenson et al. (1985). *Based on reference values from Pfeiffer et al. (2005). *Based on reference values from Christenson et al. (1985). *Defined as ≥ 10.4 µmol/L for women and as ≥ 11.4 µmol/L for men.

Table 2. Mean concentrations of As variables and urinary creatinine, by sex and cobalamin status.

| Variable | Female (n = 479) | Male (n = 299) | p-Value* | Cobalamin sufficient (n = 362) | Cobalamin deficient (n = 416) | p-Value*
|----------|-----------------|----------------|----------|-------------------------------|-------------------------------|----------|
| Water As (µg/L) | 104.8 (95.3–114.3) | 101.3 (98.6–113.0) | 0.59 | 95.7 (85.0–106.4) | 110.2 (100.0–120.3) | 0.006 |
| Urinary creatinine (mg/dL) | 58.9 (52.5–62.7) | 66.4 (60.8–72.1) | 0.12 | 62.9 (58.1–67.6) | 60.9 (56.6–65.1) | 0.49 |
| uAs (µg/L) | 141.1 (130.3–151.9) | 141.7 (125.0–158.5) | 0.18 | 136.8 (123.5–150.1) | 145.3 (132.4–158.1) | 0.16 |
| uAs/gCr | 293.3 (271.9–315.6) | 242.9 (221.2–264.4) | 0.002 | 263.5 (237.6–292.9) | 283.0 (262.7–303.2) | 0.004 |
| %As | 15.7 (15.0–16.4) | 15.0 (14.4–15.6) | 0.54 | 15.7 (14.9–15.6) | 15.2 (14.6–15.8) | 0.92 |
| %MMA | 10.7 (10.3–11.1) | 14.5 (13.9–15.1) | <0.0001 | 13.2 (12.7–13.7) | 11.2 (10.8–11.7) | <0.0001 |
| %DMA | 73.6 (72.8–74.4) | 70.5 (69.5–71.4) | <0.0001 | 71.1 (70.1–72.0) | 73.6 (72.8–74.3) | <0.0001 |
| MMA/iAs Ratio | 0.79 (0.75–0.83) | 1.07 (1.0–1.1) | <0.0001 | 0.99 (0.93–1.04) | 0.82 (0.78–0.86) | <0.0001 |
| DMA/iAs Ratio | 8.5 (8.1–8.9) | 5.7 (5.3–6.0) | <0.0001 | 6.5 (6.1–6.9) | 8.2 (7.8–8.7) | <0.0001 |

*Wilcoxon’s rank sum test for sex difference. *Wilcoxon’s rank sum test for difference by cobalamin status.
suggesting a higher As methylation capacity in women for the second methyla
tion step (Table 2). Mean concentrations of As variables by cobalamin status are also shown in Table 2. Both water As and uAs/gCr were higher among cobalamin-deficient participants. Urinary %MMA was higher and %DMA was lower among cobalamin-sufficient participants than among cobalamin-deficient participants.

In bivariate analysis, plasma cobalamin was inversely correlated with both water As and uAs/gCr (Table 3). We noted a statistically significant positive correlation between cobala
min and %MMA (r = 0.18, p < 0.0001). Both %iAs and %DMA were weakly inversely correlated with cobalamin concentration, and these associations appeared to be the same in males and females (Table 3). In linear regression models (Table 4), cobalamin was significantly inversely associated with %iAs (unstandardized regression coefficient (b) = −0.10; 95% confidence interval (CI), −0.17 to −0.02; p < 0.01) and positively associated with %MMA (b = 0.12; 95% CI, 0.05 to 0.20; p = 0.001) after adjusting for age, sex, urinary creatinine, total uAs, and BMI. These associations were stronger among females than among males, but the difference was not statistically significantly (Table 4). Cobalamin was not significantly associated with urinary %DMA. Because the influence of cobalamin on As methyla

tion could depend on an adequate supply of 5-methyl tetrahydrofolic (THF), we further examined associations between cobalamin and uAs metabolites by folate status (Table 4). Plasma cobalamin was significantly inversely associated with %iAs (b = −0.17; 95% CI, −0.30 to −0.03; p = 0.02) and positively associated with %MMA (b = 0.20; 95% CI, 0.11 to 0.30; p < 0.0001) only among folate-sufficient subjects (p-value for test of difference by folate status was 0.06 for %iAs and 0.04 for %MMA). We also examined associations between plasma cobalamin and uAs metabolites by water As concentrations ≤ 50 and > 50 µg/L (Table 4). The inverse association with %iAs was apparent only among those with water As concentrations ≤ 50 µg/L (b = −0.25; 95% CI, −0.40 to −0.09; p = 0.002); this difference was statistically significant (p = 0.005). Plasma cobalamin was significantly positively associated with %MMA in both strata of water As concentrations. Cobalamin was not significantly associated with urinary %DMA in either strata of water As.

In vitro nonenzymatic As methylation by methylcobalamin. Incubation of arsenite with methylcobalamin in the presence of GSH resulted in the production of MMA (Figure 2). The amount of MMA increased with increasing molar ratios of CH$_3$B$_12$:arsenite and was highest with a molar ratio of 3:5 at a pH of 7.4. The amount of MMA also increased as the incubation period increased but appeared to plateau at approximately 9 hr. Very small amounts of DMA (i.e., < 0.05%) were also detected after 6–9 hr of incubation (data not shown).

Discussion

This study complements our previous work in Bangladesh, in which we have investigated the contributions of folate and fHcyS to variations in As methylation. In our study region in Araihazar, Bangladesh, the prevalence of cobalamin deficiency (8% of men and 13% of women < 151 pmol/L) is much lower than that of folate deficiency (57% of men and 39% of women < 9 nmoL/L) (Gamble et al. 2005a). The current cross-sectional study, in which we oversampled cobalamin-deficient individuals, allowed us to examine a full range of plasma cobalamin values in relation to As metabolism. Our results showed that plasma cobalamin was inversely associated with %iAs and positively associated with %MMA in urine. Both of these associations were stronger among folate-sufficient individuals.

In addition to our previous cross-sectional study (Gamble et al. 2005b), six other studies (Chung et al. 2006; Hall et al. 2007; Heck et al. 2007; Li et al. 2008; Pilsner et al. 2009; Zablotska et al. 2008) have examined the role of cobalamin in relation to As metabolism or susceptibility to the health effects of As exposure. In our previous cross-sectional study of 1,016 Bangladeshi adults (Heck et al. 2007), we reported that dietary cobalamin intake as calculated from a food frequency questionnaire was inversely associated with %iAs and positively associated with %MMA in urine. These associations persisted after adjusting for age, sex, total caloric intake, cigarette smoking, and total uAsS and are consistent with our current observations. In our recent cross-sectional study among 10,628 Bangladeshi adults chronically exposed to As, dietary cobalamin intake was not significantly associated with the odds of having skin lesions.

Table 3. Spearman’s correlation coefficients between cobalamin and As variables by sex.

| Variable                  | Total sample | M (n = 299) | F (n = 479) |
|---------------------------|--------------|-------------|-------------|
| Water As (µg/L)           | −0.08*       | −0.04       | −0.11       |
| Urinary creatinine (mg/dL)| 0.07         | 0.09        | 0.04        |
| uAs (µg/L)                | −0.03        | 0.04        | −0.08       |
| uAs/gCr                   | −0.12*       | −0.02       | −0.09       |
| Urinary %iAs              | −0.06        | −0.02       | −0.09       |
| Urinary %MMA              | 0.18*#       | 0.10        | 0.18*       |
| Urinary %DMA              | −0.08*       | −0.05       | −0.05       |

*p < 0.05. **p < 0.01. †p < 0.001. ‡p < 0.0001.

Table 4. Parameter estimates for effect of increasing log plasma cobalamin on percentage of uAsS metabolites.

|                  | %iAs          | %MMA         | %DMA         |
|------------------|---------------|--------------|--------------|
|                  | Parameter estimate (95% CI) | p-Value | Parameter estimate (95% CI) | p-Value | Parameter estimate (95% CI) | p-Value |
| Total (n = 796)  | −0.10 (−0.17 to −0.02) | 0.03       | 0.17 (0.05 to 0.30) | 0.0001 | 0.12 (0.11 to 0.23) | 0.001 |
| Females (n = 471) | −0.12 (−0.22 to −0.01) | 0.03       | 0.17 (0.07 to 0.26) | 0.0009 | 0.12 (0.05 to 0.20) | 0.001 |
| Males (n = 325)  | −0.06 (−0.17 to 0.05) | 0.31       | 0.05 (0.07 to 0.17) | 0.38  | 0.12 (0.06 to 0.18) | 0.38  |
| Folate sufficient (n = 385) | −0.17 (−0.30 to −0.03) | 0.02       | 0.20 (0.11 to 0.30) | <0.0001 | 0.10 (0.02 to 0.18) | 0.0001 |
| Folate deficient (n = 401) | −0.22 (−0.09 to 0.05) | 0.06       | 0.05 (0.07 to 0.16) | 0.41  | 0.05 (0.02 to 0.10) | 0.05  |
| Water As < 50 µg/L (n = 310) | −0.25 (−0.40 to −0.09) | 0.002      | 0.16 (0.02 to 0.30) | 0.03  | 0.005 (0.02 to 0.18) | 0.02  |
| Water As > 50 µg/L (n = 456) | −0.0008 (−0.08 to 0.07) | 0.98       | 0.10 (0.02 to 0.18) | 0.02  | 0.005 (0.02 to 0.18) | 0.02  |

*p-Value for the test of significance of the parameter estimate. †p-Value from the Wald test for differences in stratum-specific estimates (male vs. female, folate sufficient vs. deficient, and water As < 50 µg/L vs. > 50 µg/L). *Adjusted for log age (continuous), sex, log urinary creatinine (continuous), log total uAs (continuous), and log BMI (continuous). †Adjusted for all variables in (*) except sex.
Our findings of stronger associations between plasma cobalamin and uAs metabolites among women than among men are likely explained by the higher prevalence of cobalamin deficiency among these women. The modification of the associations between cobalamin and uAs metabolites by folate status is consistent with the role of 5-methyl THF as the methyl donor for the conversion of homocysteine to methionine by methionine synthase, the reaction for which cobalamin is a required cofactor and 5-methyl THF is a cosubstrate; methionine synthase activity is reduced when 5-methyl THF is limiting. It is unclear, however, why in the presence of adequate folate, cobalamin is not associated with increased %DMA. We hypothesize that cobalamin facilitates the first methylation step and that the reaction has a tendency to stop at MMA, although the underlying mechanism cannot be determined from our data. Interestingly, Drobna et al. (2006) found that shRNA silencing of AS3MT in HepG2 cells reduced the percentage of 73 iAs III methylated to DMA from 53% to 11%, whereas the synthesis of MMA actually increased from 10% to 13% of total 73 iAs. Similarly, AS3MT knockout mice synthesize as much, if not more, MMA than wild-type mice (Drobna et al. 2009). These results strongly suggest the presence of an AS3MT-independent mechanism for MMA biosynthesis and prompted us to conduct an in vitro experiment.

Our in vitro methylcobalamin experiments, modeled after those of Zakaryan and Aposhian (1999), produced similar findings and support their suggestion that small amounts of MMA may be formed nonenzymatically. Our experiments differed from those of Zakaryan and Aposhian in that we examined the effect of pH and the molar ratio of CH3B12:arsenite on the production of MMA, and we omitted SAM, a possible source of methyl groups, from the reaction mixture. Not surprisingly, our results indicated that increasing the molar ratio of CH3B12:arsenite resulted in increased production of MMA. We also observed that the nonenzymatic methylation of arsenite by methylcobalamin was increased at pH levels closer to the normal pH of blood and other tissues than at pH 7.8. Whether the findings from our in vitro experiment, along with those of Zakaryan and Aposhian (1999), are relevant in vivo deserves further investigation.

The implications of our finding that cobalamin is associated with %MMA, but not with %DMA, are not entirely clear. However, a higher %MMA and lower %DMA in urine has been associated with increased risks of As-related skin lesions, skin cancer, bladder cancer, and peripheral vascular disease (Ahsan et al. 2007; Chen et al. 2003a, 2003b; Huang et al. 2008; Tseng 2007). These studies have measured %MMA IV–III in urine because of the difficulty of preserving the valence state. However, MMA IV is relatively nontoxic, whereas MMAIII is highly toxic (Styblo et al. 2002; Wang et al. 2007). Tseng (2007) suggested that the associations between MMA in urine and disease risks may actually be due to MMAIII, which is not stable and is readily oxidized to MMAV. Our observed positive association between cobalamin and total %MMA in urine, taken together with recent evidence regarding the toxicity of MMAIII, suggests that any influence of cobalamin on As-associated disease risk may not be favorable.

We measured plasma total cobalamin rather than methylmalonic acid or homocysteine, two measurements that may be more sensitive indicators of cobalamin deficiency (Clarke et al. 2007; Obeid and Herrmann 2007). Although we may underestimate the prevalence of cobalamin deficiency, the statistical associations between cobalamin and uAs metabolites are dependent upon the underestimation of individuals, not upon absolute concentrations of cobalamin. The interpretation of our findings is also limited by the expression of uAs metabolites as percentages, given that these values are relative to each other. However, meaningful patterns of association can still be detected when the data are expressed in this manner. We also did not have a measure of plasma choline or betaine, which may both be relevant sources of methyl groups for As methylation. In addition, we were not able to adjust for possible confounding by dietary intake of other nutrients that may influence As metabolism and also be correlated with cobalamin intake (such as methionine or creatine).

In summary, among this group of Bangladeshi adults with a high prevalence of cobalamin deficiency, cobalamin appeared to facilitate the methylation of iAs to MMA, particularly among women and among folate-sufficient individuals. The results of our in vitro experiment confirm that incubation of iAs with methylcobalamin can generate small amounts of MMA nonenzymatically. This finding is consistent with in vivo cell culture studies suggesting that alternative mechanisms for biosynthesis of MMA, independent of AS3MT, likely exist (Drobna et al. 2006, 2009). Given the recent evidence suggesting that MMAIII may be the most toxic As metabolite, our findings raise the possibility that, in contrast to folate, cobalamin supplementation may not favorably influence As metabolism.

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