Folic acid supplementation lowers blood arsenic[1–3]

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ABSTRACT

Background: Chronic arsenic exposure currently affects >100 million persons worldwide. Methylation of ingested inorganic arsenic (InAs) to monomethylarsonic (MMAs) and dimethylarsinic (DMAs) acids relies on folate-dependent one-carbon metabolism and facilitates urinary arsenic elimination.

Objective: We hypothesized that folic acid supplementation to arsenic-exposed Bangladeshi adults would increase arsenic methylation and thereby lower total blood arsenic.

Design: In this randomized, double-blind, placebo-controlled trial, we evaluated blood concentrations of total arsenic, InAs, MMAs, and DMAs in 130 participants with low plasma folate (<9 nmol/L) before and after 12 wk of supplementation with folic acid (400 μg/d) or placebo.

Results: MMAs in blood was reduced by a mean ± SE of 22.24 ± 2.86% in the folic acid supplementation group and by 1.24 ± 3.59% in the placebe group ($P < 0.0001$). There was no change in DMAs in blood; DMAs is rapidly excreted in urine as evidenced by an increase in urinary DMAs ($P = 0.0099$). Total blood arsenic was reduced by 13.62% in the folic acid supplementation group and by 2.49% in the placebo group ($P = 0.0199$).

Conclusions: Folic acid supplementation to participants with low plasma concentrations of folate lowered blood arsenic concentrations, primarily by decreasing blood MMAs and increasing urinary DMAs. Therapeutic strategies to facilitate arsenic methylation, particularly in populations with folate deficiency or hyperhomocysteinemia or both, may lower blood arsenic concentrations and thereby contribute to the prevention of arsenic-induced illnesses. *Am J Clin Nutr* 2007;86:1202–9.

KEY WORDS Folic acid, folate deficiency, homocysteine, *S*-adenosylmethionine, SAM, creatinine, arsenic, monomethylarsonic acid, dimethylarsinic acid, blood arsenic

INTRODUCTION

Arsenic is the most common source of metal or metalloid poisoning. Current estimates indicate that as many as 100 million persons in India, Bangladesh, Vietnam, Cambodia, and Nepal are drinking water with arsenic concentrations up to 100 times the World Health Organization guideline of 10 μg/L (1, 2). Other countries, including China, Taiwan, Mexico, Chile, and the United States, also have naturally occurring arsenic in groundwater that is used for drinking. In Bangladesh, as part of a milestone effort to reduce infant mortality associated with diarrheal disease in the 1960s, UNICEF and other nongovernmental organizations encouraged a massive shift from drinking microbially contaminated surface water to drinking groundwater accessed by tube wells. Twenty years later, it was discovered that roughly one-half of these wells contained high concentrations of arsenic (2). Chronic exposure to arsenic is associated with a greater risk of cancers of the skin, bladder, lung, and liver and of noncancer outcomes such as stroke (3), ischemic heart disease (4), and neurologic sequelae in adults (5) and children (6). Clinical manifestations of arsenic toxicity vary considerably between persons and populations, and poor nutritional status is thought to confer greater susceptibility (7).

In Bangladesh, the predominant form of arsenic in drinking water is trivalent inorganic arsenic ($InAs^{III}$). $InAs^{III}$ undergoes hepatic methylation with *S*-adenosylmethionine (SAM), a product of one-carbon metabolism, as the methyl donor. One-carbon metabolism consists of a series of oxidation-reduction reactions that provide carbon groups for the synthesis of nucleic acids and for the generation of methyl groups used in a multitude of important transmethylation reactions (8). Methylation of $InAs^{III}$ yields methylarsonic acid (MMAs) and *S*-adenosylhomocysteine. *S*-adenosylhomocysteine hydrolysis generates homocysteine and adenosine. MMAs is reduced to $MMAs^{III}$ before acquiring a second methyl group from SAM-yielding DMAs ($DMAs^{V}$). Several enzymes have been identified that are capable of catalyzing these transitions (9–12). The regeneration of SAM and the removal of the product inhibitor, *S*-adenosylhomocysteine, are achieved largely by downstream remethylation of homocysteine by methionine synthase using *N*5-methyltetrahydrofolate as a cosubstrate. Because methylation of arsenic facilitates urinary arsenic elimination, methylation has been considered to be a detoxification process.

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A high prevalence of hyperhomocysteinemia and folate deficiency among Bangladeshi adults, particularly men, was previously documented by our group (13) in association with a reduced capacity to methylate arsenic (14). In a recent, randomized, double-blind, placebo controlled trial of folic acid supplementation, we analyzed total arsenic and arsenic metabolites in urine and showed that folic acid supplementation resulted in an increase in the proportion of total urinary arsenic excreted as DMAs (%DMAs) and a reduction in %MMAs and %InAs in urine (15). We therefore hypothesized that the facilitation of arsenic methylation with folic acid supplementation lowers total blood arsenic concentrations. Recent methodologic advances have permitted us to test this hypothesis in banked specimens from the same randomized trial by measuring total arsenic and arsenic metabolites in blood, where concentrations are an order of magnitude lower than those in urine. We recently reported that blood arsenic is a biomarker of arsenic exposure and is directly associated with the risk of arsenic-induced skin lesions (16). Thus, lowering blood arsenic with folic acid has the therapeutic potential to reduce the risk of arsenic-induced illnesses.

SUBJECTS AND METHODS

The Nutritional Influences on Arsenic Toxicity (NIAT) study previously reported on the prevalence of folate deficiency and hyperhomocysteinemia in Bangladesh and on the effects of this folic acid intervention on arsenic metabolites in urine. The NIAT study works in collaboration with the Health Effects of Arsenic Longitudinal Study, a prospective cohort study of 11 746 adults, from which the current sample is derived (17).

The region

The study site, located ≈30 km east of Dhaka, Bangladesh, is a 25-km^2 region within the thana of Araihazar (a thana is an administrative unit, or subdivision, of one of the 64 districts of Bangladesh). Our data on socioeconomic status and those of Columbia University's Center for International Earth Science Information Network (18) indicate that this region is not particularly poor by Bangladeshi standards.

Participants

A cross-sectional study of 1650 participants, reported elsewhere (13), determined the prevalence of folate and cobalamin deficiencies and of hyperhomocysteinemia, and identified a pool of participants with low plasma folate for recruitment into the folic acid intervention study (15). The 200 participants enrolled in the folic acid supplementation trial were a random selection from the 550 participants who were in the lowest tertile for plasma folate in the cross-sectional study. Participants were excluded if they were pregnant or cobalamin deficient (vitamin B-12 $\leq$ 185 pmol/L) or if they were taking vitamin supplements. Study participants selected for this study (n = 130) are a subset of the 194 participants who completed the randomized, controlled folic acid supplementation trial (NIAT; 15). The subset includes all participants in the trial who had detectable concentrations of InAs, MMAs, and DMAs in blood. Aside from arsenic exposure, subjects selected for this study did not differ according to baseline characteristics in the NIAT study from which they were selected (data not shown).

Oral informed consent was obtained by our Bangladeshi field staff physicians, who read an approved assent form to the study participants. Ethical approval was provided by the institutional review boards of Columbia Presbyterian Medical Center and the Bangladesh Medical Research Council.

Study design and field work

As previously described (15), field staff teams visited the homes of potential subjects to assess eligibility and to invite those eligible to enroll in the folic acid intervention study. Eligible persons who consented to participate were randomly assigned to receive folic acid (400 μg/d) or placebo. One bottle containing 100 tablets of folic acid or placebo was assigned to each person. After blood and urine samples were collected, the field staff observed while each participant took a folic acid or placebo tablet. Field staff retained the bottles of folic acid or placebo tablets and returned daily to participants' homes to witness compliance.

Of the 200 study participants enrolled, 6 were unavailable to meet with our field staff to receive the folic acid or placebo tablet on a daily basis and were therefore dropped from the study. Two of these 6 were female and 4 were male; 3 had been randomly assigned to the folic acid group and 3 to the placebo group. There were no adverse events. A supply of multivitamins was provided to all participants on completion of the study.

Sample collection and handling

Plasma samples were obtained by venipuncture at the time of recruitment and after the 12-wk intervention. Blood was collected into heparin-containing evacuated tubes that were placed in IsoRack cool packs (Brinkmann Instruments, Westbury, NY) designed to maintain samples at 0 °C for 6 h. Within 4 h, samples were transported in hand-carried coolers to our local laboratory, which is situated at our field clinic in Araihazar. Samples were spun at 3000 × g for 10 min at 4 °C, and plasma was separated from cells. Aliquots of plasma and whole blood were stored at −80 °C and shipped, frozen on dry ice, to Columbia University for analysis. Urine samples were collected in 50-mL acid-washed polypropylene tubes. These were kept in portable coolers, frozen at −20 °C within 4 h, and similarly shipped on dry ice.

Analytic techniques

Water arsenic

Water arsenic concentrations of the tube well at each participant's home were obtained during a survey of all wells in the study region (19). Samples were analyzed at Columbia University's Lamont Doherty Earth Observatory by graphite furnace atomic absorption (GFAA) spectrometry by using a spectrometer (Z8200; Hitachi, Tokyo, Japan) that has a detection limit of 5 μg/L. Samples found to have nondetectable arsenic by GFAA were reanalyzed by inductively coupled mass spectrometry (ICP-MS) that has a detection limit of 0.1 μg/L (20).

Blood arsenic metabolites and total blood arsenic

Whole-blood specimens were digested according to method of Csanaky and Gregus (21). Frozen samples were thawed and mixed with 0.1 volume of 5.5% Triton X-100 (Fischer Scientific, Fairlawn, NJ). After the addition of 0.1 volume of 150 mmol aqueous mercury chloride/L and incubation on ice for 1 min, samples were deproteinized with one volume of 0.66 mol ice-cold $HClO_4$/L and centrifuged for 10 min at 4000 RPM and at

4 °C (CentraCL3R; ThermoElectron Corp, Needham Heights, MA). The supernatant was mixed with mobile phase, injected onto the HPLC column, and detected by ICP-MS with dynamic reaction cell (DRC) technology (ICP-MS-DRC). Calibration standards of a mixture of arsenic metabolites were similarly processed. ICP-MS-DRC coupled to HPLC separates and detects 6 arsenic metabolites chromatographically separated by anion exchange with the use of a PRP-X100 column (Hamilton Co, Reno, NV). The mobile phase is 10 mmol ammonium nitrate–ammonium phosphate/L, pH 9.1 (22). Arsenocholine (AsC), arsenobetaine (AsB), MMAs, DMAs, As^{III}, and As^{V} are detectable with great precision, even in blood samples with total arsenic concentrations as low as 3 μg/L. We report InAs as total InAs because As^{III} can oxidize to As^{V} during sample transport and preparation. However, we note that most of the InAs in blood appeared as As^{III}. We use 2 types of quality-control samples. We have blood samples purchased from the Institut de Santé Publique du Québec (Québec, Canada) that have known concentrations of 23 different elements, including arsenic. We also have our own set of blood samples spiked with all 5 metabolites—AsC, As^{III}, DMAs, MMAs, and As^{V}—at 3 different levels to cover the expected range of arsenic in unspiked samples. We ran both sets of quality-control samples at the beginning of every working day and throughout the day, after every 10 samples.

Total urinary arsenic

Total urinary arsenic concentrations were measured by using GFAA spectrometry in a graphite furnace system (AAnalyst 600; Perkin-Elmer, Shelton, CT) in the Columbia University Trace Metals Core Laboratory, as described previously (23). Our laboratory participates in a quality-control program for total urinary arsenic that is coordinated by Philippe Weber at the Quebec Toxicology Center (Québec, Canada). During the course of the present study, intraclass correlation coefficients between our laboratory's values and the samples calibrated at Weber's laboratory were 0.99. Urinary creatinine was analyzed by using a method based on the Jaffe reaction (24).

Urinary arsenic metabolites

Urinary arsenic metabolites were speciated by using a method described by Reuter et al (22), which employs HPLC separation of AsB, AsC, As^{V}, As^{III}, total MMAs ($MMAs^{III}$ and MMAs co-elute in a single peak), and total DMAs, followed by detection by ICP-MS-DRC. We report InAs as total InAs because As^{III} in urine can oxidize to As^{V} during sample transport and preparation.

Plasma folate and vitamin B-12

Plasma folate and total cobalamin were analyzed by radioimmunoassay (Quantaphase II; Bio-Rad Laboratories, Richmond, CA) as described previously (13). The within- and between-day CVs for folate were 3% and 11%, respectively, and those for cobalamin were 4% and 8%, respectively.

Plasma total homocysteine concentrations

Plasma total homocysteine concentrations were measured by using HPLC with fluorescence detection according to the method described by Pfeiffer et al (25). The within- and between-day CVs for total homocysteine were 5 and 8%, respectively.

Statistical analysis

Our primary outcome variables were total blood arsenic and InAs, MMAs, and DMAs (μg/L) in blood; these were measured at baseline and at the end of treatment. Urinary arsenic metabolites were measured at 3 time points: baseline, day 7, and the last day of treatment (day 84).

Descriptive statistics were calculated to describe the sample characteristics. Treatment group differences were detected by using the chi-square test for categorical variables and Wilcoxon's rank-sum test for continuous variables. We used *t* tests to detect within-subject changes in quantitative variables from before treatment to after treatment. Linear regression analysis was employed to assess the treatment effect on continuous outcomes, such as within-person change or percentage change in blood metabolites, with and without control for covariates. These analyses were conducted with SAS software (version 9.1.3; SAS Institute Inc, Cary, NC).

RESULTS

Characteristics of the study population are presented in **Table 1**. There were no significant between-group differences in baseline plasma folate, cobalamin, or total homocysteine; sex distribution; age; height; weight; BMI; total urinary arsenic; urinary arsenic metabolites; total blood arsenic; blood arsenic metabolites; water arenic; or sociodemographic variables such as education or type of house. Water arsenic concentrations of the tube well at each participant's home ranged from 0.4 to 435 μg/L; 93% of the participants had concentrations $>$ 10 μg/L, the World Health Organization guideline level, and 75% had concentrations $>$ 50 μg/L, the Bangladeshi standard.

Decline in blood arsenic

Total blood arsenic concentrations declined from a preintervention mean ± SE of 9.86 ± 0.62 μg/L to 8.20 ± 0.50 μg/L after the intervention ($P < 0.0001$) in the folic acid group, whereas the placebo group had a modest, nonsignificant decline from 9.59 ± 0.63 μg/L to 9.14 ± 0.61 μg/L ($P = 0.10$) (**Table 2**). The treatment effect on the change in total blood arsenic did not differ after adjustment for age, BMI, and sex (data not shown).

The effect of folic acid compared with that of placebo on the percentage change in total blood arsenic concentrations is shown in **Figure 1**. The percentage change was defined as the difference between posttreatment and pretreatment blood arsenic, expressed as a percentage of the pretreatment measure. A regimen of folic acid supplementation at a dose of 400 μg/d (ie, the US recommended dietary allowance) for 12 wk resulted in a decline in total blood arsenic of 13.62 ± 2.87% and a decline of 2.49 ± 3.25% in the placebo group ($P = 0.0199$). These percentage declines in total blood arsenic were essentially identical after further adjustment for age, sex, and BMI (13.1 ± 3.1% and 2.1 ± 2.1%, respectively). In all analyses, data were analyzed according to the intent-to-treat principle; however, postintervention plasma folate values for 2 subjects in the placebo group were high at >60 nmol/L, which suggests that those 2 subjects received folic acid rather than placebo; and plasma folate for 1 participant in the folic acid group was low at 7 nmol/L, which suggests that this participant did not receive folic acid. Repeating the analysis with the actual treatment resulted in percentage

TABLE 1
Baseline characteristics of the participants in a clinical trial of folic acid intervention[1]

Baseline variables	Folic acid group ($n = 68$)	Placebo group ($n = 62$)
Male	42.6 (29/68)[2]	43.6 (27/62)
Arsenic-induced skin lesions	10.6 (7/66)	8.2 (5/61)
Cigarette smoking	37.9 (25/66)	41.0 (25/61)
Use of betelnut	38.2 (26/68)	45.2 (28/62)
House type [*n* (%)]		
Thatched or other	3 (4.4)	5 (8.1)
Corrugated tin	62 (91.2)	53 (85.5)
Semi-pakka	3 (4.4)	4 (6.5)
Number of children [*n* (%)]		
0	30 (44.1)	29 (46.8)
1–2	16 (23.5)	12 (19.4)
≥3	22 (32.4)	21 (33.9)
Education [*n* (%)]		
0 y	32 (47.1)	32 (47.1)
1–5 y	24 (35.1)	24 (35.1)
>5 y	12 (17.7)	12 (17.7)
Age (y)	38.2 ± 10.9[3]	37.2 ± 9.2
Education (y)	2.9 ± 3.3	2.9 ± 3.2
Weight (kg)	46.4 ± 7.9	45.7 ± 7.1
Height (cm)	155.5 ± 8.6	154.6 ± 8.4
BMI (kg/m^2)	19.2 ± 2.9	19.1 ± 2.7
Children (*n*)	1.7 ± 1.9	1.7 ± 1.9
Plasma folate (nmol/L)	8.8 ± 5.3	8.0 ± 3.1
Vitamin B-12 (pmol/L)	285.6 ± 103.6	281.3 ± 125.1
Homocysteine (μmol/L)	10.5 ± 4.1	10.4 ± 5.9
Water arsenic (μg/L)	125.8 ± 90.4	135.0 ± 117.1
Urinary arsenic		
(μg/L)	181.3 ± 139.0	175.4 ± 140.6
(μg/Cr)	377.5 ± 271.6	352.6 ± 220.9
Urinary creatinine (mg/DL)	55.7 ± 48.5	53.2 ± 34.9

[1] Semi-pakka, a construction with half in concrete and half in another material (eg, corrugated tin). *P* values from chi-square test and Wilcoxon's rank-sum test for treatment group differences in categorical and quantitative variables showed no significant group differences in any variables.
[2] Percentage; *n*/total *n* available in parentheses (all such values).
[3] $\bar{x}$ ± SD (all such values).

changes in blood arsenic of 13.6% and 1.4% in the folic acid and placebo groups, respectively ($P = 0.006$).

Change in arsenic metabolites in blood and urine over time

Decline in blood inorganic arsenic

On average, InAs was reduced by a mean ± SD of 0.58 ± 0.91 μg/L after intervention in the folic acid group and by 0.32 ± 0.73 μg/L in the placebo group. The difference between groups was not significant. Concentrations of blood InAs declined on average by 18.54% (Table 2) and 10.61% in the folic acid and placebo groups, respectively. The between-group difference was borderline significant ($P = 0.0754$). We note that exposure to InAs was not altered over the course of the study, although subsequent mitigation strategies have reduced exposure in our study region (26).

Decline in blood monomethylarsonic acids

The reduction in total blood arsenic in the folic acid group was largely due to the decline in MMAs in blood (bMMAs). Whereas the mean ± SD decline in total blood arsenic was 1.67 ± 2.90 μg/L ($P < 0.0001$), that in bMMAs was 1.08 ± 1.46 μg/L ($P < 0.0001$). In the placebo group, the corresponding differences were 0.45 ± 2.59 μg/L ($P = 0.18$) in total blood arsenic and 0.19 ± 1.19 μg/L ($P = 0.53$) in bMMAs.

As shown in Table 2, the estimated mean ± SE within-person percentage decline in bMMAs was 22.24 ± 2.86% in the folic acid group and 1.24 ± 3.59% in the placebo group ($P < 0.0001$). Average bMMAs was reduced from 4.13 ± 0.31 μg/L before intervention to 3.04 ± 0.22 μg/L after intervention ($P < 0.0001$) in the folic acid group and from 3.97 ± 0.32 μg/L to 3.78 ± 0.29 μg/L in the placebo group ($P = 0.10$).

The pattern of the decline in bMMAs from before intervention to after intervention is shown in **Figure 2**. Concentrations of bMMAs ranged from 1 to 15 μg/L. Eighty-six percent of participants in the folic acid group and 45% of participants in the placebo group experienced a decline in bMMAs. Greater declines were observed in participants with higher baseline bMMAs than in those with lower baseline bMMAs.

Increase in dimethylarsinic acids in urine

Urine samples were collected at 3 time points: baseline, after 1 wk, and after 12 wk. Urinary arsenic variables are expressed per gram of urinary creatinine to adjust for variations in hydration status. Whereas the concentration of DMAs in blood at 12 wk did not differ significantly from that at baseline, we observed an increase in DMAs in urine of 20.84 μg/g Cr ($P = 0.03$) or 10.16% ($P = 0.0007$) after 1 wk in the folic acid group and a decline of 0.59 μg/g Cr or 0.33% ($P = 0.9$) in the placebo group (group difference: $P = 0.0207$ and 0.0099 for μg/g Cr and percentage change, respectively). By week 12, the difference in the percentage change in DMAs between groups was no longer significant. As we have previously reported (15), urinary creatinine increased in response to folic acid supplementation, likely because of enhanced synthesis of creatine (a major consumer of methyl groups). Thus, we cannot rule out the possibility that the increase in creatinine may have masked a sustained increase in urinary DMAs.

Distributions of arsenic metabolites in blood and in urine

To assess the extent to which the distribution of arsenic metabolites in urine reflects that in blood, we calculated Spearman correlation coefficients for total arsenic and arsenic metabolites between blood (μg/L) and urine (μg/g Cr) at the baseline visit. In each case, the correlations were very high, ranging from 0.68 to 0.81 ($P < 0.0001$ for all metabolites). However, when the arsenic metabolites were expressed as a percentage of total arsenic, the correlations between blood and urine, although still highly significant, were somewhat less strong (Spearman correlations: 0.32–0.44; $P < 0.001$). The proportions of InAs, MMAs, and DMAs in blood are compared with those in urine in 130 participants at the baseline visit (**Figure 3**). Expressed as a percentage of total arsenic, the concentrations of InAs and MMAs are higher and the concentrations of DMAs are lower in blood than in urine.

DISCUSSION

Folic acid supplementation lowered total blood arsenic concentrations by increasing the methylation of InAs and MMAs to DMAs, which is rapidly excreted in urine. This was evidenced by

TABLE 2
Absolute values and estimated percentage within-person change from baseline in arsenic metabolites in blood and in urine[1]

			P	
	Folic acid group ($n = 68$)	Placebo group ($n = 62$)	Group difference[2]	Two-factor model[3]
Total blood arsenic				
Baseline	9.86 ± 0.62[4]	9.59 ± 0.63		
Week 12	8.20 ± 0.50	9.14 ± 0.61		0.0113[5]
Blood arsenic metabolites (μg/L)				
Blood InAs				
Baseline	2.49 ± 0.16	2.45 ± 0.15		0.7465[6]
Week 12	1.90 ± 0.12	2.12 ± 0.14		< 0.0001[7]
Percentage change since baseline (%)	−18.54 ± 3.60	−10.61 ± 3.38	0.0754	
Blood MMAs				
Baseline	4.13 ± 0.31	3.97 ± 0.32		
Week 12	3.04 ± 0.22	3.78 ± 0.29		<0.0001[5]
Percentage change since baseline (%)	−22.25 ± 2.86	−1.24 ± 3.59	<0.0001	
Blood DMAs				
Baseline	3.24 ± 0.17	3.17 ± 0.19		0.6847[6]
Week 12	3.24 ± 0.19	3.24 ± 0.20		0.9779[7]
Percentage change since baseline (%)	1.62 ± 3.30	5.61 ± 4.01	0.8103	
Urinary arsenic/creatinine				
Baseline	377.45 ± 32.93	352.64 ± 28.04		
Week 1	402.88 ± 42.14	348.86 ± 29.32		0.6590[6]
Week 12	318.76 ± 25.23	316.14 ± 24.19		<0.0001[7]
Urinary arsenic metabolites (μg/g Cr)				
InAs/Cr				
Baseline	63.75 ± 9.97	46.97 ± 4.11		—[8]
Week 1	48.03 ± 5.39	47.00 ± 5.07		NS
Week 12	34.03 ± 3.67	39.45 ± 4.89		0.0263
Percentage change since baseline (%)				
Week 1	2.36 ± 10.59	8.69 ± 7.51	NS	
Week 12	−30.21 ± 4.94	−3.35 ± 12.22	0.0330	
MMAs/Cr				
Baseline	44.27 ± 4.22	45.32 ± 4.76		—[9]
Week 1	39.48 ± 3.54	46.78 ± 5.09		0.0214
Week 12	30.94 ± 2.89	37.17 ± 3.36		0.0038
Percentage change since baseline (%)				
Week 1	−6.69 ± 3.04	8.14 ± 4.73	0.0021	
Week 12	−24.78 ± 4.46	−4.53 ± 5.12	0.0017	
DMAs/Cr				
Baseline	252.85 ± 22.21	240.84 ± 20.72		—[10]
Week 1	273.29 ± 25.12	240.25 ± 21.76		0.0207
Week 12	245.70 ± 20.61	230.00 ± 17.90		NS
Percentage change since baseline (%)				
Week 1	10.16 ± 2.86	0.33 ± 2.97	0.0099	
Week 12	5.26 ± 5.44	2.05 ± 5.89	NS	

[1] InAs, inorganic arsenic; MMAs, monomethylarsonic acid; DMAs, dimethylarsinic acid; Cr, creatinine. No group differences were observed in any of the baseline measures, $P > 0.52$. Data on urinary As metabolites are a subset of those previously reported as InAs, MMA, and DMA; here they are shown in μg/g Cr units and as percentage change for purposes of comparison to blood arsenic variables in the current subset having blood arsenic variables.

[2] Wilcoxon's test.

[3] Linear models with repeated measures were applied to log-transformed outcome variables of urinary arsenic and metabolites (μg/Cr) as well as blood arsenic and metabolites (μg/L). The models include predictors for treatment group and time with or without treatment-by-time interaction. The generalized estimating equations approach was used in estimation of model parameters to take into account within-subject correlation in repeatedly measured outcomes. The *P* value for treatment-by-time interaction(s) or for main effects was calculated based on score tests.

[4] $\bar{x} \pm$ SE (all such values).

[5] Treatment-by-time interaction.

[6] Treatment effect.

[7] Time effect.

[8–10] The overall *P* value for the interaction with 3 time points and 2 treatments in the 2-factor models: [8] $P = 0.0892$ for InAs/Cr, [9] $P = 0.0055$ for MMAs/Cr, [10] $P = 0.0705$ for DMAs/Cr.

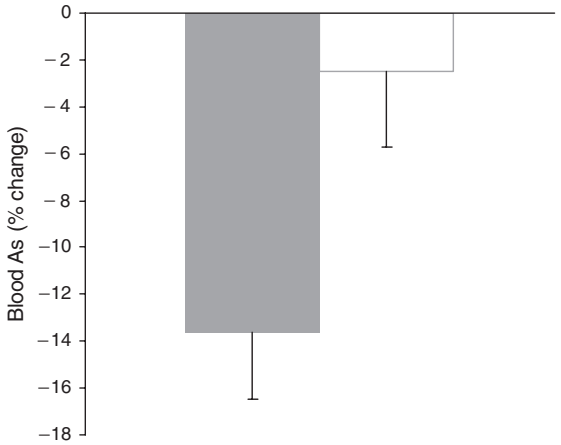

FIGURE 1. Mean (±SE) percentage change in total blood arsenic from baseline after supplementation for 12 wk with folic acid (■) (n = 68) or placebo (□) (n = 62) (P = 0.02).

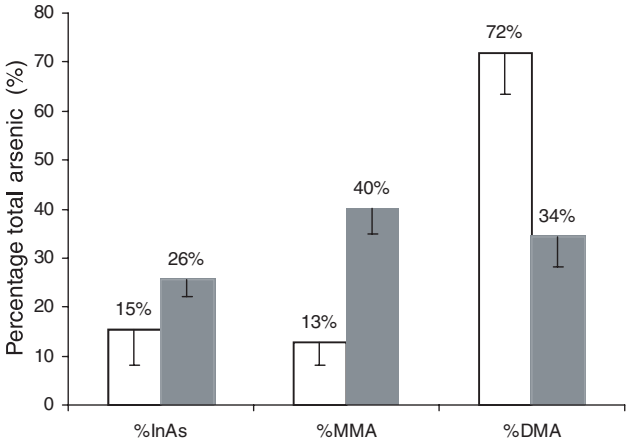

FIGURE 3. Mean (±SD) percentage of arsenic in blood and in urine at baseline in 130 participants. □, the percentage of total urinary arsenic present as inorganic arsenic (InAs), monomethylarsonic acid (MMAs), and dimethylarsinic acid (DMAs) in urine; ■, the percentage of total blood arsenic as InAs, MMAs, and DMAs in blood. Plotted values are the mean ± SD for 130 participants at baseline.

reductions in the concentrations of InAs and MMAs in blood and an increase in the concentration of DMAs in urine.

Arsenic methylation has long been considered to be a detoxification mechanism. In the 1980s, dietary methyl donor deficiency was shown to significantly decrease total urinary arsenic excretion and to significantly increase retention of arsenic in tissues in animal models (27, 28); this reflects the longer half-life and greater chemical reactivity of the InAs species. Arsenicals in blood are eliminated with a 3-component exponential decay pattern. The first and quantitatively most substantial half-life for InAs is ≈1–2 d, the second is 9.5 d, and the third is 38 d; these values were determined in humans (29). Similar patterns for InAs have been observed in rabbits and hamsters. Although the half-life of MMAs and DMAs in humans has not been determined, their initial half-life of elimination in hamsters is very short (7.4 and 5.6 h, respectively) (30), which indicates the importance of arsenic methylation in the facilitation of elimination.

The influence of arsenic methylation on arsenic toxicity has, however, been under intense investigation in recent years. Landmark work by Styblo et al (31) and Petrick et al (32, 33) in 2000 found $MMAs^{III}$ to be the most toxic metabolite, both in vitro and in vivo. $DMAs^{III}$ has been reported to have DNA-nicking activity (34), but the extent of in vivo formation of $DMAs^{III}$ is not known. A study by Valenzuela et al (35) indicated that $DMAs^{III}$ may represent a significant proportion of total urinary arsenic. However, the potential for artifact is high, because $DMAs^{III}$ is highly unstable and difficult to measure in aqueous solutions, and it has been found to co-elute with a sulfur-containing arsenical, potentially a breakdown product of arseno-protein compounds (36). Moreover, $DMAs^{III}$ is very easily oxidized to $DMAs^{V}$ (37–39). Data suggesting that $DMAs^{V}$ is a bladder carcinogen in rats (40) have been, to some extent, discounted in terms of human relevance because of the extraordinarily high doses employed (41).

Arsenite toxicity is largely attributable to its ability to react with critical sulfhydryl groups of many enzymes. It is important that the complex of arsenic with a given protein bestows selectivity to the biological effects of arsenic (42), and arsenic metabolites differ in their protein-binding capacity: arsenite has 3 coordination sites, $MMAs^{III}$ has 2, and $DMAs^{III}$ has only one (43). Because a stable structure forms only when arsenic complexes with 2 sulfhydryl groups in a single protein, the stability

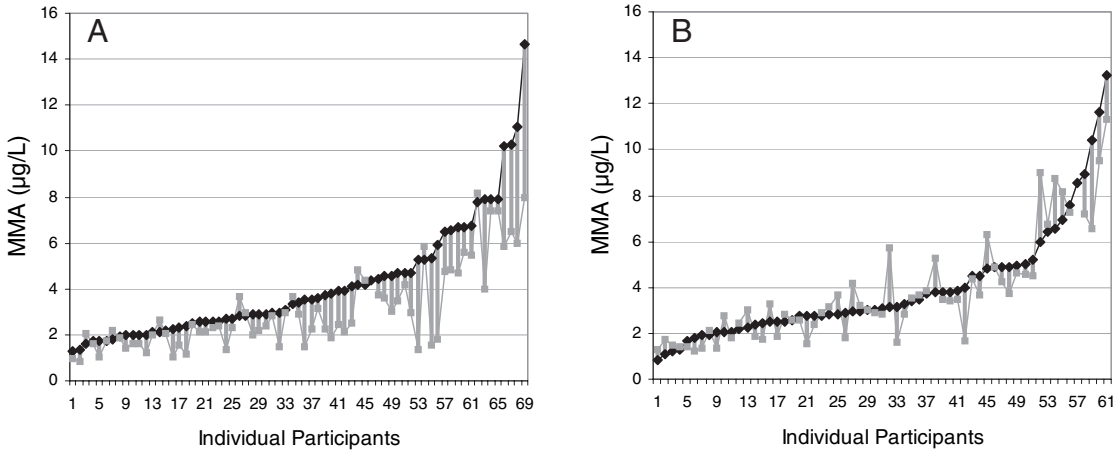

FIGURE 2. Baseline blood concentrations of monomethylarsonic acid (MMAs; μg/L) for each participant (♦) in the folic acid (A) and placebo (B) groups. — (smoothed by sorting on baseline MMAs), the range of MMAs at baseline (1–15 μg/L); ▨, each participant's corresponding blood MMAs after the 12-wk intervention. Rising and falling vertical bars indicate each participant's increase or decrease, respectively, in blood MMAs from that participant's baseline.

and specificity of binding of DMA^{III} with monothiols is less than that formed between $InAs^{III}$ and $MMAs^{III}$ with dithiols (42).

In human populations, case-control studies indicate that persons with relatively low proportions of urinary $DMAs^{(III+V)}$ and high proportions of $MMAs^{(III+V)}$ are at greater risk of arsenic-related health outcomes, including skin lesions, skin and bladder cancers, and cardiovascular disease (44–48). By chance, 8 of the participants in the current study had arsenic-induced skin lesions. These participants had significantly higher proportions of MMAs and lower proportions of DMAs in blood than did the participants without skin lesions (data not shown). The weight of the human evidence favors the consensus that incomplete methylation of arsenic to DMAs confers increased susceptibility to multiple adverse health outcomes.

Investigation of methylation of arsenic in human populations has been reliant until now almost entirely on the measurement of arsenic metabolites in urine, where concentrations are an order of magnitude higher than in blood and therefore are readily detectable with the use of conventional GFAA spectrometry–based methods. However, the untested, underlying assumption has been that arsenic metabolites in urine reflect arsenic metabolites in blood.

Assessment of total arsenic and arsenic metabolites in urine is complicated by several factors. First, the concentration of urine varies as a function of hydration status, which necessitates that metabolites be expressed per gram of creatinine. This expression is inherently problematic because urinary creatinine *1*) is itself a significant predictor of %InAs and %DMAs in urine (14, 15); *2*) is biochemically linked to one-carbon metabolism insofar as its biosynthesis consumes more methyl groups than do all other SAM-dependent methylation reactions combined (49); *3*) differs by muscle mass and consequently by age and sex; and *4*) is influenced by other factors, such as renal function, physical activity, and diet (50). To circumvent some of these issues, arsenic metabolites in urine often are expressed as a percentage of total urinary arsenic or as ratios, eg, the ratio of InAs to MMAs or that of MMAs to DMAs. Each of these expressions, however, limits the interpretation of the data: as one metabolite increases, another consequently decreases, and it becomes difficult if not impossible to decipher which metabolite was biologically important. The correlations between arsenic metabolites in blood and those in urine were all quite strong. Nevertheless, the problems associated with urinary analyses are considerable, and our understanding of the effects of folic acid supplementation on arsenic metabolism and elimination was greatly enhanced by the assessment of arsenic metabolites in blood.

In conclusion, folic acid supplementation of persons with marginal folate nutritional status decreases total blood arsenic by decreasing bInAs and bMMAs and increasing urinary DMAs. This finding has particularly important implications for reducing body stores of arsenic after exposure has been remediated, because adverse health outcomes are known to persist for decades after exposure has ceased (51, 52). Additional studies are needed, including a dose-response study of efficacy; studies to determine whether the use of L-*N*5-methyltetrahydrofolate, methylcobalamin, or other agents may enhance efficacy; and studies to determine whether folic acid interventions can similarly lower blood arsenic when folate nutritional status is adequate. Large-scale long-term intervention studies will be required to determine whether folic acid supplementation can prevent arsenic-induced skin lesions or minimize cancer risks.

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