Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh1–3

Julia E Heck, Mary V Gamble, Yu Chen, Joseph H Graziano, Vesna Slavkovich, Faruque Parvez, John A Baron, Geoffrey R Howe, and Habibul Ahsan

ABSTRACT

Background: Inorganic arsenic (InAs) is metabolized to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), and this methylation facilitates urinary arsenic excretion. Previous studies suggest that persons with more complete methylation, characterized as greater proportions of DMA and lesser proportions of MMA and InAs in urine, have a lower risk of adverse arsenic-related health outcomes.

Objective: The purpose of this study was to examine whether the capacity to methylate arsenic differs by nutrient intake.

Design: Participants were 1016 Bangladeshi adults exposed to arsenic in drinking water. Nutrient intake was assessed with a validated food-frequency questionnaire. Multivariate regression analyses were used to examine associations of nutrients with urinary arsenic metabolite profiles.

Results: In multivariate analyses, higher intakes of cysteine, methionine, calcium, protein, and vitamin B-12 were associated with lower percentages of InAs and higher ratios of MMA to InAs in urine. Higher intakes of niacin (β = 0.22, P = 0.02) and choline (β = 0.10, P = 0.02) were associated with higher DMA-to-MMA ratios, after adjustment for age, sex, smoking, total urinary arsenic, and total energy intake.

Conclusions: Findings from the current study show the influence of multiple nutrients on arsenic methylation. In particular, this study highlights the potential importance of dietary intakes of cysteine, methionine, niacin, vitamin B-12, and choline on health effects of arsenic by modulating its metabolism. Am J Clin Nutr 2007;85:1367–74.

KEY WORDS Arsenic, nutrition, choline, methionine, cysteine, folate, methylation, Bangladesh

INTRODUCTION

Inorganic arsenic (InAs) is a recognized human carcinogen, and studies of exposed populations have found elevated risks of cancers of the skin, liver, lung, urinary tract, and bladder (1). Arsenic exposure has also been associated with cardiovascular, reproductive, developmental, and neurologic toxicity (2).

Arsenic is methylated in a S-adenosylmethionine (SAM)–dependent process. The methyl group from SAM is derived from folate or from choline or betaine. In Bangladesh, the primary form of arsenic in drinking water is arsenite (AsIII). Any arsenate (AsV) that might be present is first reduced to AsIII before methylation (3). The first methylation step is the oxidative methylation of AsIII to monomethylarsonous acid (MMAII, which can then be reduced to monomethylarsonic acid (MMAIII). MMAIII is then methylated to dimethylarsinic acid (DMAIV). It is not currently known to what extent DMAIV is then reduced to dimethylarsinous acid (DMAIV) in vivo, because this is reported to be an unstable intermediate (4). The methylation of arsenic is not complete, with some arsenic remaining as InAs and MMA.

The proportions of arsenic metabolites in urine have been used as indexes of methylation efficiency (5). In particular, the ratios of MMA to InAs and DMA to MMA were used as markers of efficiency for the first and second methylation steps, respectively. Higher proportions of MMA and InAs species in urine were associated with higher risks of cancer and noncancer health outcomes (6–8).

There is interindividual variability in the distribution of arsenic species in urine. The distributions of metabolites may be affected by host factors such as genetic variation, smoking, alcohol consumption, pregnancy, age, sex, and disease status (7, 9–14).

Nutritional factors may also be important determinants of arsenic methylation. Of particular interest are nutrients involved in the biochemical pathways involved in the generation of SAM. Inadequate intakes of folate, methionine, calories, or protein are associated with arsenic–related health effects in both humans and animals (15–20). However, only a handful of studies have investigated whether diet affects the profile of arsenic species in urine. Those studies have suggested that urinary selenium and plasma α-tocopherol, folate, and cysteine may be associated with proportions of arsenic species in urine (9, 21, 22) and that dietary protein, iron, zinc, and niacin are associated with urinary MMA

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and DMA (23). Little is known as to how these and other nutrients may affect persons chronically exposed to high concentrations of arsenic. The purpose of the current study was to examine the association of dietary intake of nutrients involved in one-carbon metabolism, including folate, methionine, cysteine, niacin, pyridoxal phosphate (vitamin B-6), cobalamin (vitamin B-12), choline, and betaine, with the profile of arsenic metabolites in urine.

SUBJECTS AND METHODS

Health Effects of Arsenic Longitudinal Study

In Bangladesh, where most residents obtain drinking water through hand-pumped tubewells, recent surveys have discovered arsenic contamination in wells in much of the country. The scale of this exposure is considerable, with an estimated one-fourth to one-half of wells in Bangladesh contaminated at concentrations above the maximum standard (10 \mu g/L) recommended by the World Health Organization (3). Participants for the current investigation were participants in the Effects of Arsenic Longitudinal Study (HEALS) project, a prospective cohort study established in Araihazar, Bangladesh, in 2000. Detailed methods for HEALS were described elsewhere (24). Briefly, the purpose of the study was to examine the carcinogenic and other health effects of exposure to arsenic in drinking water. Araihazar was selected as the study site because of the wide variation of concentrations of arsenic in groundwater within that region (25).

Eligibility criteria included marriage (to increase stability of residence) and residence in the same bari (cluster of homes) for at least 5 years. Between 22 October 2000 and 19 May 2002, 14,828 potential participants meeting the eligibility criteria were identified. Nineteen percent of those eligible (n = 2778) were not at home during study visits. Of the 12,050 who were available and approached, 11,746 (97.5%) participated. A previous study tested the temporal stability of water arsenic concentrations in wells in the region and found little variation during a 3-year period (25). The participants underwent standardized interviews that provided information on demographic characteristics, diet, and well use. In addition, all participants underwent systematic physical examinations by trained physicians to detect the presence of any arsenic-related signs or symptoms. Informed consent was obtained, and the study was approved by institutional review boards at Columbia University and in Bangladesh.

At the time of selection of the case and control subjects for the present study, the recruitment of cohort members was completed, and roster lists of 11,746 overall and 11,224 participants with urine samples were created. We randomly selected a 10% sample of the 11,224 cohort members who provided urine samples (n = 1123) for a further analysis of urinary arsenic metabolites. Of the 1123 subjects, 12 did not complete a physical examination and 70 had prevalent skin lesions. Because cases with skin lesions may have altered urinary arsenic profiles (10, 11), the current study included only the unaffected participants, representing a random sample of the total cohort free of skin lesions. The 1041 subjects did not differ significantly from the remainder of nondiseased persons in the cohort by age, sex, water arsenic exposure, or mean concentration of urinary arsenic (P = 0.20–0.94).

Dietary measurement

After conducting focus group discussions to learn the range of the diet of this population, we developed a food-frequency questionnaire (FFQ), which was subsequently validated (26). In the FFQ, participants were asked to describe average food intakes during the year before the interview. The micronutrients of primary interest were those involved in one-carbon metabolism, several of which have been found in prior investigations to be associated with the presence of skin lesions or with change in urinary arsenic (15, 19, 23); these micronutrients included folate, methionine, cysteine, choline, betaine, and vitamins B-6 and B-12. Other nutrients, including niacin, iron, calcium, ascorbic acid, protein, riboflavin, thiamin, fiber, and vitamins A and E, were reported to be protective against arsenic toxicity in other studies and were included in analyses for comparison purposes. The choline content of foods was calculated as the sum of the amounts of choline, phosphocholine, phosphatidylcholine, glycerophosphocholine, and sphingomyelin. Betaine, a metabolite of choline, was examined independently. All nutrients were adjusted for total energy intake with the use of the residual method (27), and intakes were divided into quartiles for analysis. Information on nutrient composition was derived from the US Department of Agriculture National Nutrient Database for Standard Reference (28). Selenium may also be an important factor in prevention of health effects from arsenic and also in arsenic metabolism. However, the selenium content of foods depends highly on selenium content in soil, and the soil content of selenium in this region is not well known. As such, accurate dietary measurement of selenium could not be ascertained, and this nutrient was not included in our analyses.

Measurement of total urinary arsenic and urinary arsenic metabolites

A spot urine sample was collected at the time of the physician examination. Total urinary arsenic concentrations were measured with the use of graphite furnace atomic absorption spectrometry (Perkin-Elmer, Shelton, CT) (25). Metabolites were speciated by using HPLC separation of arsenobetaine (AsB), arsenocholine (AsC), AsV, AsIII, MMA, and DMA, followed by detection by inductively coupled–mass spectrometry (29). Because AsIII can oxidize to AsV during sample storage and preparation, we report total InAs (AsIII + AsV).

Fish is an important part of the Bangladeshi diet. Seafood products contain the nontoxic arsenic metabolites AsB and AsC. These species accounted for ≈3% of the total urinary arsenic in our participants. AsB and AsC were excluded from final estimates of total urinary arsenic. Thus, after subtraction of AsB and AsC, we calculated the proportions of InAs, MMA (MMAIII + MMAIV), and DMA (DMAIII + DMAIV). We defined the efficiency of the first and second methylation steps as the ratio of MMA to InAs and the ratio of DMA to MMA, respectively.

Statistical analyses

After computing descriptive summary statistics, we used analysis of variance to compare proportions of urinary arsenic metabolites between participants categorized according to sex, age, body mass index (BMI; in kg/m²), smoking, and total urinary arsenic. To improve normality, log transformation of urinary arsenic values was done before inclusion in regression analyses. Data were analyzed with the use of SAS 9.1 (SAS Institute, Cary, NC).

Our primary outcomes were the proportions of urinary InAs, MMA, and DMA, and the ratios of the first (MMA/InAs) and second (DMA/MMA) methylation products. Generalized linear
regression was used to determine the associations of nutrient intakes with these outcomes. Variables considered for inclusion in models were age, sex, smoking, total caloric intake, BMI, and urinary arsenic. All were included in final models except BMI, which had little effect on outcomes.

RESULTS

After omission of 25 participants with missing information, the final sample size was 1016 participants (412 men and 604 women) aged between 18 and 65 y (Table 1). The participants were exposed to arsenic concentrations in drinking water, ranging from 0.1 to 749 μg/L. As indicated in Table 1, the percentage of MMA was higher in men, older persons, participants with lower BMI, smokers, and participants exposed to higher well arsenic concentrations (>150 μg/L). The percentage of DMA had the opposite association, because it was higher in women, participants with BMI > 25, nonsmokers, and participants exposed to lower well arsenic concentrations. The percentage of InAs declined with age and was lower among participants exposed to the lowest well arsenic concentrations and participants with the highest urinary arsenic. In this cohort, age, sex, and smoking were interrelated, because men in the cohort were older (mean age: 40.5 y) than were women (mean age: 33.9 y), and men were much more likely to smoke (68.7% of male participants compared with 4.0% of female participants).

MMA:InAs was higher in men and increased with age. Smokers and those exposed to the lowest concentrations of well water arsenic also had greater MMA:InAs. Women had higher DMA: MMA, as did younger adults, participants with higher BMI (>25), nonsmokers, and participants exposed to the lowest well water concentrations of arsenic.

Bivariate associations between dietary intakes of nutrients and urinary arsenic profiles are shown in Table 2. The percentage of InAs in urine was inversely associated with intakes of cysteine, methionine, vitamin B-12, calcium, iron, protein, and riboflavin, and it was directly associated with intake of betaine. The percentage of MMA was directly associated with dietary intakes of cysteine, folate, methionine, calcium, iron, protein, riboflavin, and vitamins A, B-12, and E, whereas greater niacin intake was associated with a decreased percentage of MMA. Nutrient intakes had no significant association with percentage of DMA.

Multivariate analyses of the associations between dietary nutrients and proportions of urinary arsenic metabolites after control for age, sex, total caloric intake, current cigarette smoking, and total urinary arsenic are shown in Table 3. Dietary intakes of cysteine, methionine, vitamin B-12, calcium, iron, protein, riboflavin, and vitamins A, B-12, and E, whereas greater niacin intake was associated with a decreased percentage of MMA. Nutrient intakes had no significant association with percentage of DMA.
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<th>Nutrient</th>
<th>Energy-adjusted intake</th>
<th>Percentage InAs</th>
<th>P for trend&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Percentage MMA&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>P for trend&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>Percentage DMA&lt;sup&gt;§&lt;/sup&gt;</th>
<th>P for trend&lt;sup&gt;§&lt;/sup&gt;</th>
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<td><strong>Folate-methionine pathway nutrients</strong></td>
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<td>Betaine (mg)&lt;sup&gt;†&lt;/sup&gt;</td>
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<td>Q1</td>
<td>0.47–7.70</td>
<td>15.31 ± 7.96&lt;sup&gt;§&lt;/sup&gt;</td>
<td>0.03</td>
<td>12.93 ± 5.06</td>
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<td>12.64 ± 5.16</td>
<td>71.06 ± 8.58</td>
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<td>Q3</td>
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<td>16.29 ± 7.89</td>
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<td>70.68 ± 9.09</td>
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<td>Q4</td>
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<td>12.53 ± 4.64</td>
<td>70.77 ± 7.67</td>
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<td>15.18 ± 7.01</td>
<td>0.6</td>
<td>12.32 ± 5.33</td>
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<td>71.34 ± 8.71</td>
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<td>Cysteine (mg)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>501.1–767.4</td>
<td>16.09 ± 6.43</td>
<td>0.001</td>
<td>12.08 ± 4.82</td>
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<td>70.99 ± 8.18</td>
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<td>Folate (μg)&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>44.5–217.9</td>
<td>16.19 ± 6.82</td>
<td>0.1</td>
<td>11.88 ± 4.57</td>
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<td>71.09 ± 8.50</td>
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<td>Methionine (mg)&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>907.5–1420.8</td>
<td>15.92 ± 6.87</td>
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<td>11.96 ± 4.90</td>
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<td>71.17 ± 8.60</td>
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<td>Vitamin B-6 (mg)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>2.13–3.24</td>
<td>15.30 ± 6.72</td>
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<td>12.56 ± 5.10</td>
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<td>71.25 ± 8.10</td>
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<td>Vitamin B-12 (μg)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>0.01–1.03</td>
<td>16.28 ± 7.02</td>
<td>0.02</td>
<td>11.89 ± 5.02</td>
<td>0.0003</td>
<td>71.00 ± 8.52</td>
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<td>Other nutrients</td>
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<td>Ascorbic acid (mg)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>2.1–61.2</td>
<td>15.39 ± 6.33</td>
<td>0.6</td>
<td>12.59 ± 5.22</td>
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<td>71.32 ± 7.98</td>
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<td>Calcium (mg)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>35.9–304.56</td>
<td>16.34 ± 6.65</td>
<td>0.001</td>
<td>11.97 ± 4.87</td>
<td>&lt;0.0001</td>
<td>70.85 ± 8.32</td>
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<td>Fiber (g)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>21.7–40.21</td>
<td>15.00 ± 6.58</td>
<td>0.5</td>
<td>12.34 ± 5.03</td>
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<td>71.88 ± 8.56</td>
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<td>Iron (mg)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>4.70–11.20</td>
<td>16.50 ± 6.91</td>
<td>0.02</td>
<td>11.97 ± 4.52</td>
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<td>70.75 ± 8.64</td>
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<td>Niacin (mg)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>20.7–30.3</td>
<td>15.39 ± 7.27</td>
<td>0.5</td>
<td>13.45 ± 5.09</td>
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<td>Protein (g)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>43.6–64.87</td>
<td>16.28 ± 6.63</td>
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<td>12.29 ± 4.91</td>
<td>0.0004</td>
<td>70.63 ± 8.42</td>
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<td>Riboflavin (mg)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>0.310–0.781</td>
<td>15.95 ± 6.35</td>
<td>0.009</td>
<td>11.78 ± 4.56</td>
<td>&lt;0.0001</td>
<td>71.33 ± 7.93</td>
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DISCUSSION

In our population of Bangladeshi adults with long-term exposure to elevated concentrations of arsenic in drinking water, we found associations between the distribution of urinary arsenic metabolites and dietary intake of several nutrients. These findings highlight the importance of nutrition in potentially protecting against the health effects of arsenic exposure in this population. The apparent association of some nutrients with reducing urinary arsenic metabolism depends on adequate intakes of multiple nutrients, including folate and vitamins B-6 and B-12 (39).

Methionine and folate are important dietary sources of methyl groups. Even minor dietary differences in these nutrients can contribute to variation in SAM, SAH, and homocysteine concentrations (40). The importance of dietary sources of methyl groups was previously shown in studies that linked low intakes of these nutrients with the development of different types of cancer (41, 42). Similarly, in rats, methyl-deficient diets decrease concentrations of SAM and increase SAH and were associated with the development of liver tumors (40).

The null effect seen with dietary folate differs from previous research by our group, which showed that plasma concentrations of folate were positively correlated with the percentage of DMA in urine and inversely correlated with percentages of MMA and InAs (21, 35). In those studies, folate nutritional status was assessed by plasma measures of folate concentrations. In contrast, the current study assessed the naturally occurring folate content of foods before cooking. Although the FFQ validation study with the current study assessed the naturally occurring folate content in urine and inversely correlated with percentages of MMA and positively correlated with the percentage of DMA (40). The importance of dietary sources of methyl groups is particularly notable because of the direct association between InAs and cancer risk (6, 7, 34). A study in Taiwan found that a greater percentage of MMA and percentage of InAs predicted a 3- to 5-fold increased risk of skin lesions (34).

The current study is the largest investigation to date to examine associations between dietary nutrients and urinary arsenic metabolites among healthy participants. The strengths of the investigation include the large sample size, the wide variability in water arsenic concentrations, and the minimal refusal rates in the overall cohort. Because study eligibility required residence at the same address for a minimum of 5 y, the study population had stable exposure to arsenic in well water before recruitment.

The current study complements our recent studies of plasma folate, homocysteine, cysteine, and cobalamin in our Bangladeshi cohort (35, 36). Those studies found a high prevalence of hyperhomocysteinemia in this population, which was associated with reduced methylation of MMA to DMA. The methylation of MMA to DMA may be inhibited by S-adenosylhomocysteine (SAH), which becomes elevated in association with hyperhomocysteinemia (36, 37). SAH, a byproduct of SAM methylation reactions, is known to be a potent inhibitor of most methyltransferase enzymes (38). SAH remains tightly bound to methyltransferases and is only removed if the pathway is pulled forward by downstream removal of homocysteine. SAH hydrolysis depends on adequate intake of several nutrients, including folate and vitamins B-6 and B-12 (39).

The findings of the current study also differ from the studies by Gamble et al (21, 35) with regard to vitamin B-12, likely because those studies excluded participants with low plasma concentrations of vitamin B-12. The prevalence of vitamin B-12 deficiency (<151 nmol/L plasma) in our cohort was previously estimated to be 8% among men and 13% among women (36). One-carbon metabolism depends on adequate intakes of multiple nutrients,
and the results of this investigation must be interpreted in light of the overall poor diet quality of many study participants.

Inadequate methylation of arsenic is a particular concern because it is associated with greater arsenic toxicity. In animals, methyl-deficient diets have resulted in reduced arsenic methylation and higher tissue concentrations of arsenic (16, 43). Methylation was traditionally considered a detoxification process because of the lesser tissue damage and enhanced urinary excretion of MMA and DMA compared with arsenite. Some investigators have suggested that the trivalent metabolites (MMA\textsuperscript{3} and DMA\textsuperscript{3}) may be more toxic than the pentavalent forms of organic arsenic, suggesting that methylation itself may increase arsenic toxicity (44). Nevertheless, by facilitating urinary arsenic excretion, methylation reduces tissue exposure. Thus, complete methylation to DMA may reduce tissue exposure to arsenical compounds despite the transient production of trivalent metabolites (45).

Our findings are supported by other research that has described narrow ranges of plasma nutrients in the population, by the manner in which the samples were handled, or by differing metabolites. Findings may have been affected by the signs (48, 49). For example, a recent study of plasma nutrients and arsenic-related cancer, possibly because of differences in study populations, methods of data collection, and research designs (48, 49). For example, a recent study of plasma nutrients found little association with case or control status in a West Bengal population (50). Findings may have been affected by the narrow ranges of plasma nutrients in the population, by the manner in which the samples were handled, or by differing metabolism among those with prevalent disease. The current study attempted to ameliorate the latter problem by only including participants free of skin lesions.

Our study has several limitations. Demographic and dietary data were collected by self-report and are subject to the biases associated with this type of data collection. However, because no subject in our sample had a diagnosis of skin lesions, the study should not have been subject to recall bias with regard to dietary exposures.

FFQs are known to be potentially subject to biases, and the validation study of this FFQ found only moderately strong correlations (Pearson’s correlation coefficients $\geq 0.30$, corrected for within-person error) between FFQ and 2-wk food diaries for protein, riboflavin, folate, niacin, and vitamin B-12, whereas the correlations for fiber, vitamin B-6, iron, thiamin, and calcium were lower (26). We attributed this to variations in seasonal availability of foods, which would create differences between food diary and FFQ. In addition, foods associated with greater social esteem, including tea and fruit, may have been overestimated on the FFQ. As a result, there may have been nondifferential misclassification of nutrient intake, resulting in an underestimation of the association between nutrient intakes and and the results of this investigation must be interpreted in light of the overall poor diet quality of many study participants.

Inadequate methylation of arsenic is a particular concern because it is associated with greater arsenic toxicity. In animals, methyl-deficient diets have resulted in reduced arsenic methylation and higher tissue concentrations of arsenic (16, 43). Methylation was traditionally considered a detoxification process because of the lesser tissue damage and enhanced urinary excretion of MMA and DMA compared with arsenite. Some investigators have suggested that the trivalent metabolites (MMA\textsuperscript{3} and DMA\textsuperscript{3}) may be more toxic than the pentavalent forms of organic arsenic, suggesting that methylation itself may increase arsenic toxicity (44). Nevertheless, by facilitating urinary arsenic excretion, methylation reduces tissue exposure. Thus, complete methylation to DMA may reduce tissue exposure to arsenical compounds despite the transient production of trivalent metabolites (45).

Our findings are supported by other research that has described narrow ranges of plasma nutrients in the population, by the manner in which the samples were handled, or by differing metabolites. Findings may have been affected by the signs (48, 49). For example, a recent study of plasma nutrients found little association with case or control status in a West Bengal population (50). Findings may have been affected by the narrow ranges of plasma nutrients in the population, by the manner in which the samples were handled, or by differing metabolism among those with prevalent disease. The current study attempted to ameliorate the latter problem by only including participants free of skin lesions.

Our study has several limitations. Demographic and dietary data were collected by self-report and are subject to the biases associated with this type of data collection. However, because no subject in our sample had a diagnosis of skin lesions, the study should not have been subject to recall bias with regard to dietary exposures.

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metabolite profiles. Having found significant associations between several nutrients and metabolites, we consider that the parameter values may be underestimates.

Most studies on nutrition and arsenic toxicity have found that specific nutrients appear to affect arsenic-related health outcomes. Because the participants in the current study had a high prevalence of underweight, adequate nutrition for arsenic methylation may be particularly challenging in this population. Additional studies are needed to determine whether participants with arsenic-related health effects require the same nutrients as these apparently healthy participants for more thorough methylation and whether other host factors, such as genetic variations, contribute to the nutrition-methylation relation.

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The authors’ responsibilities were as follows—JEH: conceived the study, conducted analyses, and wrote the manuscript; FP: collected and analyzed data; YC: collected and analyzed data; GRH: collected and analyzed data; HA: supervised the overall work. None of the authors has a conflict of interest.

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