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Arsenic metabolism and one-carbon metabolism at low-moderate arsenic exposure: evidence from the Strong Heart Study

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1. INTRODUCTION

Inorganic arsenic (iAs) in food and water is a major global health concern. An established carcinogen, chronic arsenic exposure also increases the risk of cardiovascular disease, respiratory disease, neurologic deficits, and diabetes.(Chen et al. 2011; Council 2001; Kuo et al. 2013; Moon et al. 2012; Naujokas et al. 2013; Tyler and Allan 2014; Wu et al. 2014) After ingestion, iAs (arsenate and arsenite) is metabolized into mono- and di-methylated arsenicals (MMA and DMA); DMA has a shorter circulating half-life and is more rapidly excreted through the urine as compared to iAs.(Aposhian and Aposhian 2006; Challenger 1951; Cullen WR 1989; Hayakawa et al. 2005; Naranmandura et al. 2006; Vahter 2002) The urinary distribution of arsenic metabolites across human populations ranges from 10–30% for iAs, 10–20% for MMA and 60–80% for DMA.(Chiou et al. 1997; Del Razo et al. 1997; Hopenhayn-Rich et al. 1996; Navas-Acien et al. 2009; Vahter 2000) Higher percentages of iAs (iAs%) and MMA (MMA%) and lower percentages of DMA (DMA%) in urine are thought to reflect a less efficient arsenic metabolism profile and have been associated with higher risk of cancer, skin lesions and cardiovascular disease.(Chen et al. 2003a; Chen et al. 2003b; Chen et al. 2005; Del Razo et al. 1997; Hsueh et al. 1997; Steinmaus et al. 2006; Wu et al. 2006; Yu et al. 2000) Conversely, higher DMA% and lower MMA% have been associated with diabetes, metabolic syndrome and higher body mass index.(Chen et al. 2012; Del Razo et al. 2011; Kuo et al. 2015; Mendez et al. 2016; Nizam et al. 2013; Wang et al. 2007) Understanding non-modifiable (genetics, sex, life-stage) and modifiable (smoking, alcohol intake, kidney function, body mass index, nutrition) determinants of arsenic metabolism is important given the role of arsenic metabolism in arsenic toxicity. (Balakrishnan et al. 2016; Council 2013; Gribble et al. 2013; Jansen et al. 2016)

Nutritional status is a major susceptibility factor for arsenic-related disease, at least in part through the impact of nutrition on one-carbon metabolism (OCM).(Council 2013) OCM, a network of interrelated biochemical reactions dependent on sufficient intake of vitamin B_2 (riboflavin), vitamin B_6, folate (vitamin B_9) and vitamin B_{12}, plays an essential role in methylation processes throughout the body, including the methylation reactions involved in arsenic metabolism (Figure 1).(Hall and Gamble 2012; Howe et al. 2014) In studies from Bangladesh, both cross-sectional (Gamble et al. 2005) and folic acid supplementation trials demonstrated (Gamble et al. 2006; Peters et al. 2015) that higher folate is associated with increased arsenic methylation efficiency, resulting in higher DMA% and lower iAs% and MMA% in urine and in reduced blood arsenic concentrations. In cross-sectional studies, greater dietary intake of vitamins B_{12} and B_2 (Heck et al. 2007) and higher plasma B_{12} (Hall et al. 2009a) have been associated with lower iAs% and higher MMA%, in Bangladeshi adults. Further, both epidemiologic (Chung et al. 2006; Pilsner et al. 2009; Zablotska et al. 2008) and experimental studies (Acharyya et al. 2015; Bhattacharjee S 2013) have reported OCM nutrients to be associated with lower risk for arsenic-related disease.

The generalizability of the OCM findings in Bangladesh to US populations with low-moderate arsenic exposure and different dietary patterns is unclear. We evaluated the association of OCM nutrients with arsenic metabolism biomarkers in the Strong Heart Study (SHS), a population-based cohort study initiated to assess cardiovascular risk factors in American Indian adults residing in Arizona, Oklahoma and North and South Dakota. We

used dietary intake estimates of B_2, B_6, folate and B_{12} as measures of OCM nutrients and percentages of urinary inorganic arsenic (iAs%) and its methylated metabolites (MMA% and DMA%), as measures of arsenic metabolism. We also modeled the complexity of both arsenic metabolism profiles and nutrition intake through the use of principal component analysis (PCA).

2. METHODS

2.1 Study Population

The SHS recruited 4,549 American Indians from 13 tribes located in Arizona, Oklahoma and North and South Dakota. Eligible participants were men and women aged 45–74 years at the baseline visit in 1989–1991. The overall participation rate was 62%. All participants provided informed consent and study protocols were approved by multiple institutional review boards, community members and The Indian Health Service. In 2016, one of the communities withdrew their consent for participating in future studies, reducing the overall sample size to 3,516. The final version of this manuscript, along with a lay summary, was sent to, and approved by, all remaining communities.

At the baseline visit (1989–1991), a random sample of 50 males and 50 females from each age decade and at each study site (n=722; 508 after excluding the community that withdrew consent) was selected to participate in a self-administered food frequency questionnaire (FFQ), which provided estimated long-term daily average intake of folate and vitamins B_2, B_6 and B_{12} in milligrams.(Committee 1989) We excluded 94 participants with missing data on urine arsenic, and 9 participants missing data on education, alcohol intake, smoking status, body mass index (BMI), estimated glomerular filtration rate (eGFR), and urine creatinine, leaving 405 participants for this study. Participants included in this study were similar to the overall study population on most variables of interest, with the exception of being slightly older than the full cohort (Supplemental Material Table S1).

2.2 Data Collection

Baseline visits included bio-specimen collection, a physical exam, and an interview-administered questionnaire. Visits were performed by trained and certified examiners according to a standardized protocol. Details have been described previously.(Lee et al. 1990)

2.2.1 Urine Arsenic Metabolites—Morning spot urine samples were collected during baseline visit in polypropylene tubes, frozen within 1 to 2 hours of collection, shipped buried in dry ice and stored at <−70°C in the Penn Medical Laboratory, MedStar Research Institute, Washington, DC.(Lee et al. 1990) The freezers have been operating under a strict quality control system to guarantee secure sample storage. For arsenic analyses, urine samples were thawed in 2009–2010, and up to 1.0 mL from each urine sample was transferred to a small vial, transported on dry ice to the Trace Element Laboratory at Graz University, Austria and stored at <−70°C until analyses.(Scheer et al. 2012)

Quality control and quality assurance methods for urine arsenic analysis have been described in detail.(Scheer et al. 2012) Urine concentrations of arsenite, arsenate, methylarsonate

(MMA) and dimethylarsinate (DMA) were measured using high performance liquid chromatography/inductively coupled plasma-mass spectrometry (HPLC/ICPMS). The limits of detection were 0.1 μg/L for arsenite, arsenate, MMA and DMA. The inter-assay coefficients of variation for arsenite and arsenate, MMA and DMA were 5.6%, 6.3%, 3.5% and 4.4% respectively. Urine arsenobetaine concentrations were measured using cation-exchange HPLC/ICPMS together with other less common arsenic cations, and the low concentration measured (median (IQR): 0.73 (0.49, 1.47) μg/L) confirmed seafood consumption in this population was infrequent. The limit of detection for urine arsenobetaine was 0.1 μg/L and the inter-assay coefficient of variation was 6.9%. No participants in this analysis had arsenic species below the limit of detection.

2.2.2 Nutrition Variables—Dietary intake of OCM-related micronutrients was measured during the baseline visit through estimated daily averages of dietary intake of vitamins $B_{2,}$ $B_{6,}$ folate and B_{12} in the past-year. These variables, as well as total caloric intake, were measured through an interviewer-administered Block 119-item food frequency questionnaire (FFQ). The Block questionnaire is one of the most widely used questionnaires with demonstrated reliability and validity.(Fretts et al. 2012) Each participant was asked how often, on average, a particular food was consumed during the past year using measures of consumption frequency and portion size adjustment.(Fretts et al. 2012)To enhance accuracy of the questionnaire in this cohort, additional questions relating to foods commonly consumed by American Indians were added.(Fretts et al. 2012) Of note, folate was calculated based on dietary intake alone as doses of folic acid supplementation were not available (only whether supplements were taken or not) and mandatory folic acid fortification was not implemented by the date of FFQ administration.

2.2.3 Other Variables—Sociodemographic (age, sex, and education) and life-style (vitamin supplementation use and drinking and smoking status) study variables were ascertained through a standardized questionnaire (separate from the FFQ) by trained and certified interviewers.(Lee et al. 1990) Height and weight measurements for BMI calculation (weight in kilometers divided by height in meters squared) were conducted during the physical exam. Urine creatinine was measured from the spot urine samples collected for arsenic analysis using an automated alkaline picrate methodology.(Lee et al. 1990) eGFR was calculated from creatinine, age and sex using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, as previously described.(Shara et al. 2012)

2.3 Statistical Analysis Methods

To evaluate arsenic metabolism, we computed the relative proportions of inorganic and methylated arsenic metabolites by dividing each metabolite concentration over the sum of those species x 100. In this way, a methylation profile was estimated for each participant, consisting of iAs%, MMA% and DMA%, totaling to 100%. We also evaluated arsenic metabolism using principal component analysis (PCA), as recently proposed.(Balakrishnan et al. 2016; Jansen et al. 2016) PCA was conducted using each arsenic species percentage, then scaling them. PCA is useful in the analysis of arsenic metabolism biomarkers because it removes the inter-dependence of the three biomarkers (iAs%, MMA% and DMA%) allowing for an improved, and potentially more biologically meaningful, interpretation of

each metabolite. Previous studies have suggested that the first arsenic metabolism principal component (As metabolism PC1) reflects the ability to produce DMA (i.e., the secondary methylation step) while the second arsenic metabolism principal component (As metabolism PC2) reflects the conversion of iAs to MMA (i.e., the primary methylation step). (Balakrishnan et al. 2016; Jansen et al. 2016) Inorganic arsenic exposure was estimated as the sum of urinary concentrations of iAs, MMA and DMA (ΣAs). ΣAs concentrations were right skewed and log-transformed for the analyses.

The analysis of dietary estimates of OCM nutrients (vitamin B_2, vitamin B_6, folate and vitamin B_{12}) requires adjustment for total caloric intake in most analyses. Because nutrient and total caloric intake variables were measured from the same questionnaire, their errors are strongly correlated. To cancel out these errors and improve the validity of energy-adjusted nutrients, nutrient density and residual methods have been suggested over simply including caloric intake as a variable in regression models.(Rhee et al. 2014) We used a residual analysis approach and regressed each log-transformed vitamin intake on log-transformed total caloric intake. We then added the mean log-transformed value of each nutrient to the nutrient residuals to create calorie-corrected nutrient variables. Because some OCM nutrients share common dietary sources and they are metabolically inter-dependent, we also used PCA to identify major PCs across log-transformed, calorie-corrected OCM nutrients.

Arsenic methylation patterns were compared across sociodemographic, health, nutrition and behavioral characteristic variable categories using Kruskal-Wallis tests. Pearson correlation coefficients were conducted to estimate univariate correlations between iAs%, MMA%, DMA% and calorie-corrected vitamins (vitamin B_2, vitamin B_6, folate and vitamin B_{12}) (Figure 2).

The mean difference (95% CI) of each of arsenic species percentages (iAs%, MMA%, DMA %) and arsenic metabolism principal component (As metabolism PC1 and As metabolism PC2) comparing the two highest to the lowest tertile of each calorie-corrected nutrient variable (vitamin B_2, vitamin B_6, folate and vitamin B_{12}) was estimated using linear regression models. We used the following progressive adjustments for known arsenic metabolism and nutrition intake determinants in our models evaluating arsenic metabolism both as individual arsenic species' percentages as well as principal components: model 1 adjusted for ΣAs (log-transformed) and urine creatinine (log-transformed); model 2 further adjusted for age (continuous), sex, study center (Arizona, Oklahoma, North/South Dakota), BMI (continuous), smoking status (never, former, current), alcohol use (never, former, current), and eGFR (continuous); model 3 further adjusted for the other OCM calorie-corrected nutrient variables. Because adjustment for all nutrients at once can be difficult due to possible collinearity, we also used OCM PCs to evaluate the association of arsenic metabolism biomarkers with independent summary variables of calorie-corrected nutrients.

We conducted multiple sensitivity analyses. First, we evaluated the potential impact of outliers in our models using resistant regression with consistent results (data not shown). Second, we evaluated an alternative method of correcting nutrients for total caloric intake (log-transformed) using adjustment in the main regression analysis instead of the residual-

based approach, with similar findings (data not shown). Third, we used specific gravity as an alternative to urinary creatinine to account for urine dilution, with consistent results (Supplemental Material Table S2). Main analyses include adjustment for urinary creatinine as it has been shown to affect arsenic metabolism, possibly through the competition for methyl groups in the synthesis of creatine, the precursor to creatinine.(Peters et al. 2015) Urinary creatinine reflects both dietary creatine intake (primarily from meat) and endogenous synthesis of creatine. In addition, we ran regressions with and without a participant with an outlying high (>100μg) value for B_{12}, again with consistent results (data not shown). We also ran regressions with and without participants reported to take folic acid or multivitamin supplements (n=9) with consistent results (data not shown). Further, to address potential concerns regarding DMA from food sources at low levels of arsenic exposure, we stratified by center due to geographical differences in exposure sources (water is a major source in Arizona and North and South Dakota, while in Oklahoma the predominant source is food). Results were similar in both areas of lower exposure (Oklahoma) and higher exposure (Arizona and Dakotas) (Supplemental Material Table S2). Finally, exploratory analyses were conducted to investigate potential pairwise interactions between each OCM nutrient. A joint categorical variable reflecting high (equal to or greater than the median) and low (below the median) intake, as well as quantitative interaction terms, of each OCM nutrient pair, was created to assess these relationships (Supplemental Material Table S3).

2.3.1 Sample Size Justification—Our sample size was fixed as our analysis was based on previously collected data. Therefore, we conducted a minimally detectable effect estimate for each of our main outcomes (iAs%, MMA% and DMA%). We used a sample size of 405 and 80% power for each calculation. For iAs%, using a mean of 8.4 and standard deviation of 4.7, we computed the minimally detectable difference to be 0.66. For MMA%, using a mean of 14.7 and standard deviation of 5.3, we computed the minimally detectable difference to be 0.70. For DMA%, using a mean of 76.9 and standard deviation of 8.8, we computed the minimally detectable difference to be 1.23.

3. RESULTS

3.1 Participant Characteristics

Median (IQR) percentages for arsenic metabolism biomarkers were 7.1 (5.2–10.7)% for iAs %, 13.7 (11.3–17.7)% for MMA%, and 78.2 (71.6–83.3)% for DMA% (Table 1). Median (IQR) for arsenic species concentrations were 0.74 (0.33, 1.58) μg/L for iAs, 1.36 (0.71, 2.65) μg/L for MMA and 7.59 (4.69, 13.52) μg/L for DMA. Median (IQR) daily intake for OCM nutrients were 1.4 (0.9–2.0) mg for vitamin B_2, 1.2 (0.8–1.9) mg for vitamin B_6, 207 (120.4–336.7) μg for folate, and 3.0 (1.7–5.7) μg for vitamin B_{12}. Several characteristics were associated with higher iAs%, higher MMA% and lower DMA%, including being male, lower BMI, current smoker and current alcohol drinker. Characteristics associated with just higher iAs% included being younger, having eGFR > 60 mL/min/1.73 m^2 and having lower intake of B_6 and folate. Compared to tertiles 1 and 3, participants in the 2nd tertile of urine creatinine had lower iAs% and higher DMA% (Table 1). Pearson correlation coefficients were moderately positive for iAs% and MMA% (0.54), and strongly negative between both

iAs% and MMA% with DMA% (−0.86 and −0.89, respectively) (Figure 2). All OCM nutrition variables were positively correlated with each other both before and after correcting for caloric intake. Calorie-corrected nutrient correlations ranged from 0.23 between vitamin B_{12} and vitamin B_6 to 0.70 between vitamin B_2 and folate. Correlations between OCM nutrients were negative with both MMA% and iAs% (ranging from −0.01 for iAs% and vitamin B_{12} to −0.13 for iAs% and vitamin B_6); and positive with DMA% (ranging from 0.03 with vitamin B_{12} to 0.13 with vitamin B_6)(Figure 2).

Variance in arsenic methylation patterns were summarized in two principal components. Arsenic metabolism principal component 1 (As PC1) explained 84.6% of the variance in arsenic species biomarkers and reflected higher DMA% and lower iAs% and MMA% (Table 2). Arsenic metabolism principal component (As PC2) explained 15.4% of the variance and reflected higher MMA% and lower iAs%, independent of DMA%. Variance in OCM nutrients was summarized in four principal components (Table 2). OCM PC1 explained 60.1% of the variance in OCM nutrients and reflected higher intake of all four B-vitamins. OCM PC2 explained 22.7% of the variance and reflected higher vitamin B_{12} intake and lower intake of vitamin B_6 and folate. OCM PC3 explained 10.3% of the variance and reflected higher folate and B_2 intake and lower intake of vitamins B_6 and vitamin B_{12}. OCM PC4 explained 6.9% of variance and reflected higher intake of vitamin B_{12} and folate and lower intake of the other nutrients.

3.2 Association of One-Carbon Metabolism Nutrients and Arsenic Metabolism Biomarkers

OCM nutrients, specifically vitamins B_6 and B_2, were associated with more efficient arsenic methylation profiles. In fully adjusted models (Table 3, Model 2), higher intake of vitamins B_2 and B_6 were associated with lower iAs%, lower MMA% and higher DMA%. Compared to tertile 1, participants in tertile 3 of vitamin B_2 intake had 1.00 (95% CI: −2.00, 0.00)% lower iAs%, 1.36 (95% CI: −2.48, −0.23)% lower MMA% and 2.36 (95% CI: 0.54, 4.18)% higher DMA%. Correspondingly, participants in tertile 3 of vitamin B_6 intake had 1.36 (95% CI: −2.35, −0.37)% lower iAs%, 1.57 (95% CI: −2.69, −0.45)% lower MMA% and 2.93 (95% CI: 1.13, 4.74)% higher DMA%. These associations, with the exception of vitamin B_2 and iAs%, remained statistically significant and similar in magnitude after further adjustment for all OCM nutrients (Table 3, Model 3).

In fully adjusted models without adjustment for other OCM nutrients (Table 3, Model 2), folate showed a similar association with arsenic metabolism biomarkers as vitamins B_2 and B_6, although they were weaker, not statistically significant, and stronger for the second tertile than the third. For example, participants in tertile 2 of folate intake compared to tertile 1 had 0.93 (95% CI: −2.06, 0.20) lower MMA% but participants in tertile 3 of folate intake compared to tertile 1 had only 0.18 (95% CI: −1.30, 0.93) lower MMA%. With further adjustment for other OCM nutrients, the association with tertile 2 of folate was attenuated, whereas tertile 3 reversed direction for all arsenic metabolism biomarkers, although the associations remained not-statistically significant. Again, using MMA% as an example, compared to tertile 1, tertile 2 was associated with 0.29 (95% CI: −1.46, 0.88) lower MMA % and tertile 3 was as associated with 1.27 (95% CI: −0.08, 2.62) greater MMA%. The

corresponding associations with vitamin B_{12} were weaker and not statistically significant in any model.

When modeling arsenic metabolism using PCA, tertile 3 of vitamins B_2 and B_6 intake were associated with higher As metabolism PC1, reflecting higher DMA% and lower iAs% and MMA% (Table 4). No significant associations were observed with any of the OCM nutrients and As metabolism PC2. In models using OCM PCs instead of the original nutrient variables, OCM PC1, reflecting higher intake of all OCM nutrients, was negatively associated with iAs% and MMA% and positively associated with DMA% and As metabolism PC1 (Table 4). OCM PC2, representing mostly high vitamin B_{12}, possibly reflecting meat intake, was not associated with arsenic methylation profiles. OCM PC3, reflecting higher intake of vitamin B_2 and folate with lower vitamin B_6, was positively associated with higher iAs% and MMA% and negatively associated with DMA% and As metabolism PC1, although none of the associations were statistically significant. OCM PC4, reflecting high folate and vitamin B_{12} and low vitamin B_6 and B_2, was positively associated with iAs% and MMA%, and negatively with DMA% and As metabolism PC1, however, only the association with MMA% was statistically significant. In analyses evaluating the joint effect of OCM nutrients in pairs, we found an independent additive association between intake of vitamins B_6 and B_2 with arsenic metabolism biomarkers. Mean DMA% was 2.76 (95% C:I 0.88, 4.65)% higher for participants with both vitamins B_6 and B_2 above (high) versus below (low) the median, 2.02 (95% CI: −0.10, 4.14)% higher for participants with only high B_6 and 1.08 (95%CI −1.09, 3.24)% higher for participants with only high B_2 (Supplemental Material Table S3). The joint association was not different from additive (p-value for interaction 0.83). For vitamin B_6 and folate, compared to participants with low intake of both vitamins, high folate intake with low B_6 was associated with 1.39 (95% CI: 0.19, 2.58)% higher iAs% and 2.58 (95% CI: −4.77, −0.93)% lower DMA% while high folate intake with high vitamin B_6 was associated with 1.03 (95% CI: −2.01, −0.05) lower iAs% and 1.65 (95% CI: −0.14, 3.44)% higher DMA%. The p-value for interaction, supporting an antagonistic interaction, between folate and B_6 was 0.01 for iAs% and 0.001 for DMA% (no interaction was found for MMA%).

4. DISCUSSION

Dietary intake of OCM nutrients was associated with urinary arsenic methylation patterns in a population of rural American Indian adult men and women exposed to low-moderate levels of inorganic arsenic from drinking water and food. In general, higher intake of B-vitamins, in particular B_2 and B_6, was associated with lower percentages of iAs and MMA and higher percentages of DMA, a profile suggested to reflect enhanced arsenic metabolism. These associations persisted for vitamins B_2 and B_6 after adjustment for sociodemographic factors, smoking, alcohol intake, BMI, and kidney function, as well as all OCM nutrients. The joint association for vitamins B_2 and B_6 was independent and not different from additive. For vitamin B_{12}, a vitamin with a relatively high intake in the study population, there was no association with arsenic metabolism biomarkers, a finding that is consistent with results from other populations with generally adequate vitamin B_{12} intake.(Gamble et al. 2005; Hall et al. 2009b) The association between folate intake and arsenic metabolism in the main analyses was not clear. In joint analyses, an antagonistic association was found between

folate and vitamin B_6, with higher folate being associated with higher DMA% and lower iAs % only in the presence of high vitamin B_6. However, these results should be interpreted with caution given the high correlation between B_6 and folate.

OCM is critical in the biosynthesis of purines and thymidylate as well as the generation of methyl groups.(Ralph Carmel 2001) OCM facilitates the generation of S-adenosylmethionine (SAM) which serves as a methyl donor for numerous substrates. These substrates are essential to multiple biological processes including cellular signaling, DNA methylation, the synthesis of proteins, lipids, hormones and carbohydrates, and arsenic metabolism.(Ralph Carmel 2001) A product of SAM-dependent methylation reactions, s-adenosylhomocysteine, is hydrolized to homosysteine (Hcys), which can be remethylated to form methionine and activated to regenerate SAM.(Ralph Carmel 2001) OCM functioning, and adequate methyl group availability, is dependent on essential nutrients including folate, vitamin B_{12}, vitamin B_6, and vitamin B_2 (Figure 1).

Numerous studies conducted in Bangladesh have characterized the relationship between OCM nutrients and arsenic metabolism in populations exposed to high levels of arsenic. Randomized controlled trials (RCTs) (Gamble et al. 2006; Peters et al. 2015) have focused on folic acid given its key role in the recruitment of methyl groups, which are required for methylation reactions in the OCM pathway.(Peters et al. 2015) Results from cross-sectional studies (Gamble et al. 2005; Hall et al. 2007; Li et al. 2008) have also shown associations between higher plasma folate and enhanced arsenic metabolism, consistent with increases in urinary DMA% and decreases in iAs% and MMA% seen in RCTs. Collectively, these studies provide strong evidence for the temporality of these associations. Cross-sectional dietary intake studies of folate and arsenic metabolism from Bangladesh(Heck et al. 2007), Mexico(Lopez-Carrillo et al. 2016) and the U.S.(Steinmaus et al. 2005), however, have been inconsistent.

Evaluation of OCM nutrients beyond folate, including vitamins B_2, B_6 and B_{12}, all of which play important roles in OCM (Figure 1), has not been as extensive, lacking evidence from RCTs. Higher vitamin B_{12} (plasma(Hall et al. 2009a) and dietary intake (Heck et al. 2007; Lopez-Carrillo et al. 2016)) was associated with lower iAs% and higher MMA% in some studies but not in studies with a lower prevalence of B_{12}-deficiency.(Gamble et al. 2005; Gamble et al. 2006; Hall et al. 2009b) These results have led to the hypothesis that increases in vitamin B_{12} may promote the first step of arsenic methylation, leading to higher MMA% and lower iAs%.(Hall et al. 2009a; Howe et al. 2014) The study reporting plasma B_{12} levels being associated with lower iAs% and higher MMA%, by design, over-selected participants having moderate- to severe-B_{12} deficiency. The predomination of the methylation of iAs to MMA over MMA to DMA may be specific to under-nourished populations exposed to high levels of arsenic, due to a competition for available methyl groups.(Howe et al. 2014) In our population, characterized by adequate or even high vitamin B_{12} intake, we found no association with arsenic metabolism. This may be because vitamin B_{12} truly does not affect arsenic metabolism in populations where vitamin B_{12} intake is high. However, it is also possible our lack of significant associations for vitamin B_{12} was due to our study being underpowered to detect an effect size of that magnitude.

Three dietary intake studies, one conducted in the U.S.(Steinmaus et al. 2005), one conducted in women from Mexico (Lopez-Carrillo et al. 2016) and one conducted in Bangladesh,(Heck et al. 2007) reported null results for vitamin B_6. The Bangladeshi dietary intake study, however, found similar associations between vitamin B_2 and arsenic metabolism as had been reported for vitamin B_{12}; higher vitamin B_2 intake was associated with lower iAs% but higher MMA%.(Heck et al. 2007) Associations for vitamin B_2 were null in the other two dietary intake studies.(Steinmaus et al. 2005)

Our analysis on the association between OCM nutrients and arsenic metabolism fills in gaps in our understanding of this relationship in a cohort relevant to the general U.S. population. The SHS population is better nourished (38% of participants from the Bangladeshi dietary intake study were underweight compared to just one participant in our study) and exposed to lower levels of arsenic than the populations studied in Bangladesh. Further, dietary patterns differ substantially between the two populations: the SHS diet generally includes fewer fresh fruits and vegetables (significant sources of vitamin B_6 and folate) (Figure 3) and more meat/protein (significant sources of B_2 and B_{12} and intake and has been associated with arsenic metabolism (Heck et al. 2007; Steinmaus et al. 2005)) than typical Bangladeshi diets.(Berg et al. 2012; Fretts et al. 2012) This is reflected in our participants' higher levels of vitamins B_{12} and B_2 and lower levels of folate and vitamin B_6 than in Bangladesh (Supplemental Material Table S4). Participants in the dietary intake study conducted among Mexican women had OCM nutrient intake patterns more similar to those in the Bangladeshi dietary intake study.(Heck et al. 2007; Lopez-Carrillo et al. 2016)

To our knowledge, only one other study has evaluated OCM nutrients and arsenic metabolism in a U.S. population.(Steinmaus et al. 2005) This study, conducted in individuals from Western Nevada and Kings County, California, exposed to arsenic levels in drinking water generally above 10 μg/L(Steinmaus et al. 2003), found no association between dietary intake of OCM nutrients and arsenic metabolism; however, the study was small (N=87), some OCM nutrients were not available (no evaluation of vitamin B_{12}), and the majority of the population was male (75%) with a large percentage having a history of bladder cancer (26%).(Steinmaus et al. 2005)

In our study, the strongest associations between vitamin intake and arsenic metabolism were for the least extensively studied OCM nutrients, vitamins B_2 and B_6. Greater intake of vitamins B_2 and B_6 were associated with higher DMA% and lower iAs% and MMA%. Our PCA results support these findings with greater intake of vitamins B_2 and B_6 also being associated with higher As metabolism PC1, which reflects lower iAs% and MMA% and higher DMA%. Because metabolism of arsenic first involves the conversion of iAs to MMA and then the conversion of MMA to DMA, As metabolism PC1 may represent overall methylation to DMA, or the secondary arsenic methylation index.

The magnitude of increases in DMA% and decreases in MMA% for participants in the third versus first tertile of intake for B_2 and B_6 were significant but modest (ranging from 1.5 to 3 percentage point differences). Translating this into clinical relevance, increasing intake of vitamin B_6 by 1.06 mg, for example, reflecting the increase in intake needed to move those at the median of the first tertile to the median of the third tertile of vitamin B_6 intake, could

result in MMA% 1–2 percentages lower. Although on the individual level, this may appear small, on the population level it could translate to significant effects on health outcomes. For example, a case-control study from Chile reported a 1.11 (1.06, 1.17) and 1.05 (1.00, 1.10) greater odds of skin cancer and bladder cancer, respectively, for each 1% increase in MMA %.(Melak et al. 2014) Further, in a Bangladesh-based trial, folate deficient participants received folic acid supplementation for 12 weeks. This resulted in a decline in MMA% in urine from 13% to 10% yet a 22% reduction in blood MMA levels and 14% reductions in participants' total blood arsenic.(Gamble et al. 2006; Gamble et al. 2007)

Further, results from our pairwise interactions suggest there is an additive independent association for high intake of vitamins B_2 and B_6 with enhanced arsenic metabolism. Our findings add evidence that vitamins B_2 and B_6, less studied B-vitamins involved in OCM, may also affect arsenic metabolism in the same direction as has been reported for folate; and perhaps, play a stronger role in arsenic metabolism than folate in well-nourished protein-sufficient populations. This finding is also supported by the OCM PC analysis. OCM PC1, reflecting higher intake of all four vitamins, was associated with lower iAs% and MMA% and higher DMA% and As metabolism PC1. However, OCM PC4, reflecting low B_2 and B_6 intake and high folate and B_{12} intake, was significantly associated with higher MMA% and non-significantly associated with higher iAs% and lower DMA%. Thus, despite high folate and B_{12} intake, low intake of B_2 and B_6 (e.g., a diet with low intake of dairy, poultry and fruit but high intake of green vegetables and certain fish) resulted in lower arsenic metabolism efficiency. This result for PC4 is consistent with the joint analysis for folate and vitamin B_6, where high folate was associated with lower arsenic metabolism efficiency in the presence of low vitamin B_6 but with higher arsenic metabolism efficiency with high B_6 intake. These findings suggest that high folate alone may not enhance arsenic metabolism in this population. Although we cannot disregard that these findings could be related to measurement error of folate nutritional status in the absence of plasma folate data, additional research is needed to evaluate if this pattern is consistent in other populations. Overall, these results suggest that, as it has been reported for plasma folate in undernourished and low-protein populations, increasing levels of OCM nutrients, specifically B_2 and B_6, can enhance arsenic methylation capacity in a well-nourished protein-sufficient population exposed to low-moderate arsenic.

In independent linear regressions analyses for folate, the stronger association with MMA% and DMA% for participants in the second tertile of folate rather than the third tertile, and the reversing of direction in tertile 3 after adjustment for other nutrients, is difficult to interpret. It could be related to measurement error from the difficulty of dietary folate measurement. We did not calculate dietary folate equivalents (DFEs), which are used to incorporate all sources of folate intake, in addition to accounting for the higher bioavailability of folic acid from supplements and fortified foods compared with naturally occurring folate in foods. (Bailey 1998; Park et al. 2013) However, sensitivity analyses excluding participants reporting vitamin supplementation use yielded consistent results, and mandatory fortification of foods with folic acid was not implemented in the United States until 1998 (almost ten years after data collection). It is also possible the patterns seen for folate and arsenic metabolism in our analysis are not consistent with other studies due to interactions with intake of other vitamins, unmeasured confounding and/or oxidation of food folates during

cooking. Studies in well-nourished populations exposed to a broad range of folate through food fortification and supplementation use, with measurements of both folate intake and biomarkers, are necessary to assess the dose-response relationship of folate intake with arsenic metabolism.

Limitations of our study include relying on self-reported dietary data from the FFQ, a dietary assessment tool that is associated with underestimates of intake, particularly energy and protein.(Mossavar-Rahmani et al. 2015; Prentice et al. 2011) Further, self-reporting intake over a full year may introduce measurement error due to recall bias. Confirmation of findings through evaluation of nutrition biomarkers would have enhanced analyses and study interpretation. Still, correlations between dietary intake estimates and plasma biomarkers of vitamin B_{12} and folate reported in a recent feeding study (r=0.63 for Vitamin B_{12} and r=0.66 for DFE) support the strength of intake estimates to reflect internal vitamin levels.(Lampe et al. 2017) In addition, data were lacking on other nutrients involved in OCM, such as methionine and choline, which would have allowed a more comprehensive understanding of the full impact of OCM and arsenic metabolism. Further, B-vitamin intake was based solely on diet, lacking information on supplements. Although we were able to confirm that seafood intake in this population was low, we were unable to adjust for other food sources of inorganic arsenic that may confound the findings, such as rice intake. Finally, since there are gene variants, for example, the common single nucleotide polymorphism in the gene for MTHFR, the 667 C→ T mutation, that lead to lower blood folate levels, the extent to which B-vitamin intake reflects internal levels of those vitamins, at least in part, is genetically determined. Additional research to evaluate the role of OCM related genetic variants and its impact on these relationships in this population would be useful.

5. CONCLUSIONS

Our study provides evidence that even at low levels of arsenic exposure and in well-nourished populations, OCM nutrient intake may affect the efficiency of arsenic metabolism. Further, our findings suggest vitamins B_6 and B_2, two previously understudied OCM vitamins, may play a stronger role in arsenic metabolism than folate in well-nourished protein-sufficient populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

As PC 1	Arsenic Metabolism Principal Component 1
As PC 2	Arsenic Metabolism Principal Component 2
BMI	Body Mass Index
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
DFE	Dietary Folate Equivalent

DMA	Dimethylarsenate
eGFR	Estimate Glomerular Filtration Rate
FFQ	Food Frequency Questionnaire
HPLC/ICPMS	High Performance Liquid Chromatography/Inductively Coupled Plasma-Mass Spectrometry
iAs	Inorganic Arsenic
MMA	Monomethylarsonate
OCM	One-Carbon Metabolism
OCM PC 1	One-Carbon Metabolism Principal Component 1
OCM PC 2	One-Carbon Metabolism Principal Component 2
OCM PC 3	One-Carbon Metabolism Principal Component 3
OCM PC4	One-Carbon Metabolism Principal Component 4
PCA	Principal Component Analysis
RCT	Randomized Controlled Trial
ΣAs	Total arsenic

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HIGHLIGHTS

- Greater intake of one-carbon metabolism (OCM) vitamins B_6 and B_2, was associated with arsenic metabolism (ASM) efficiency
- In principal component (PC) analyses, the PC reflecting greater OCM vitamin intake was also associated with enhanced ASM
- High vitamin B_6 and B_2 intake had an additive effect on ASM efficiency
- High folate with low B_6 was associated with reduced ASM, but high intake of both vitamins was associated with enhanced ASM

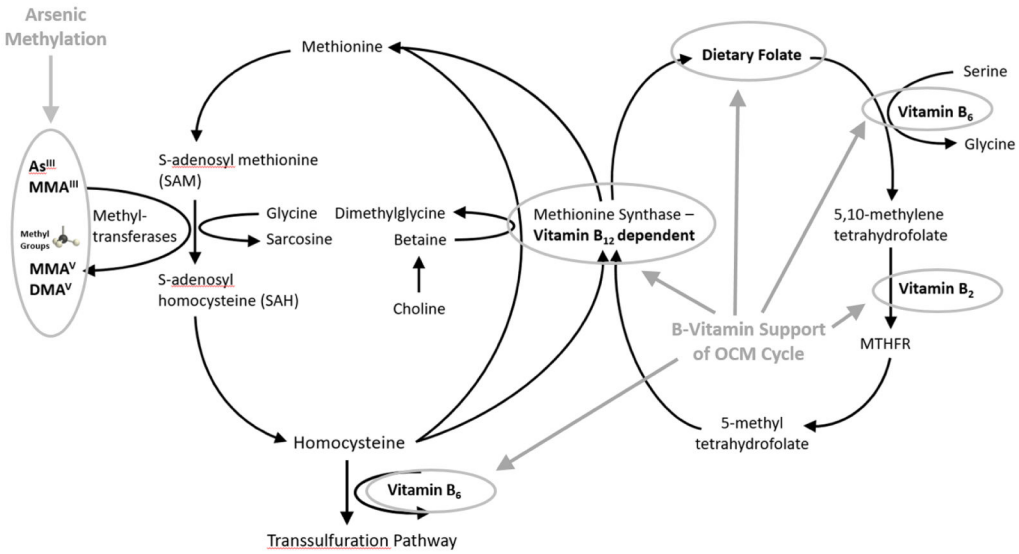

Figure 1. One-Carbon Metabolism, B-vitamins, and Arsenic Metabolism

Methionine is activated by methionine adenosyltransferase to form S-adenosylmethionine (SAM). SAM provides the methyl group for most methylation reactions in the body (including arsenic methylation reactions), after which adenosylhomocysteine (SAH) is formed as a byproduct. SAH is hydrolyzed to homocysteine, which is then used in the transsulfuration pathway, or is regenerated into methionine via betaine or B_{12} dependent pathways. Dietary folate is converted to 5-methyl tetrahydrofolate through B_6 and B_2 dependent reactions, which provides the methyl group for the regeneration of homocysteine to methionine via the B_{12} pathway.

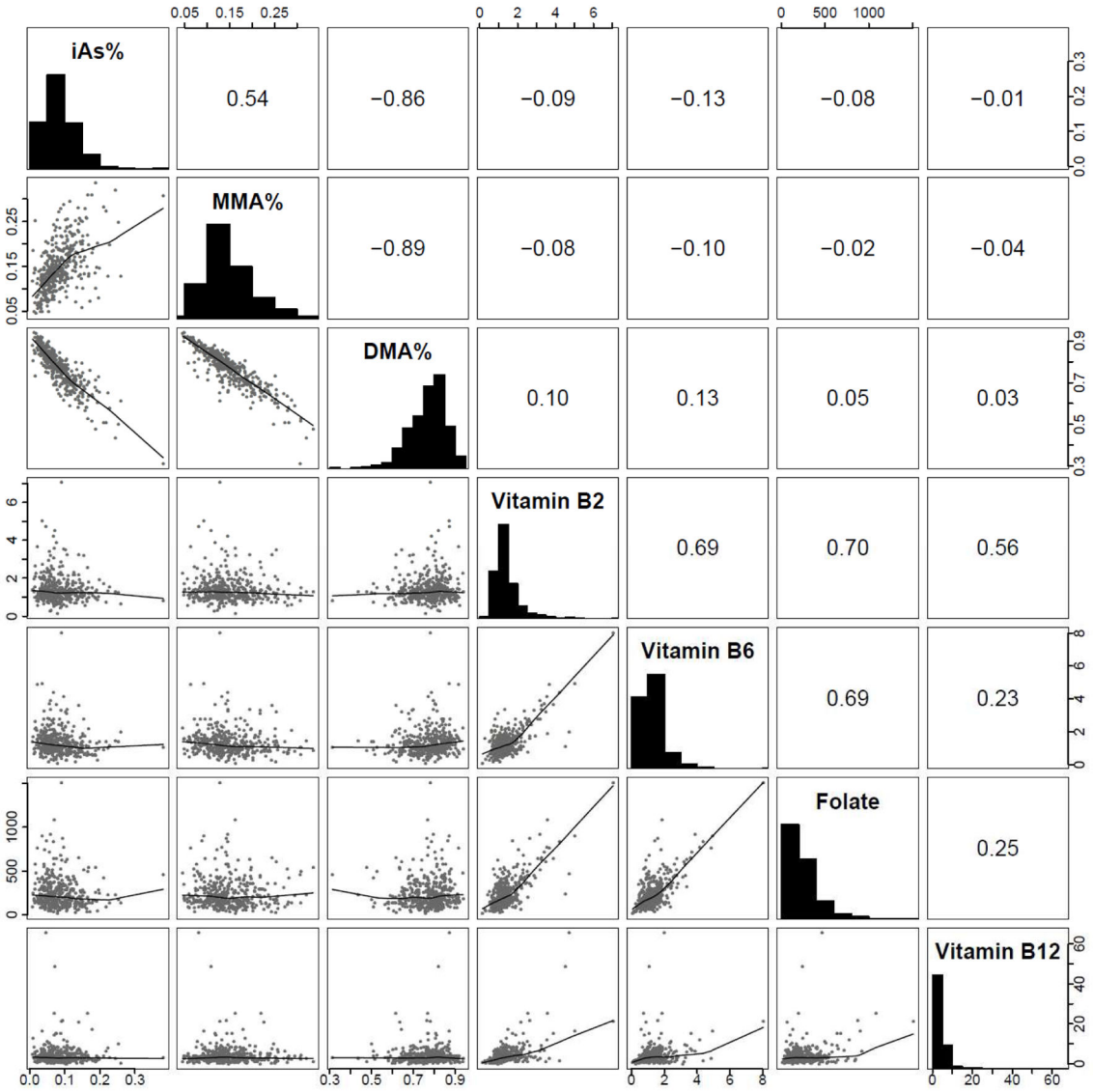

Figure 2. Pearson Correlation Matrix for Arsenic Species Percentages and Calorie-Corrected Nutrition Variables

Correlations and histograms of arsenic species percentages and calorie-corrected, using a residual analysis method of calorie-adjustment, nutrition variables (B_{12} outlier not included).

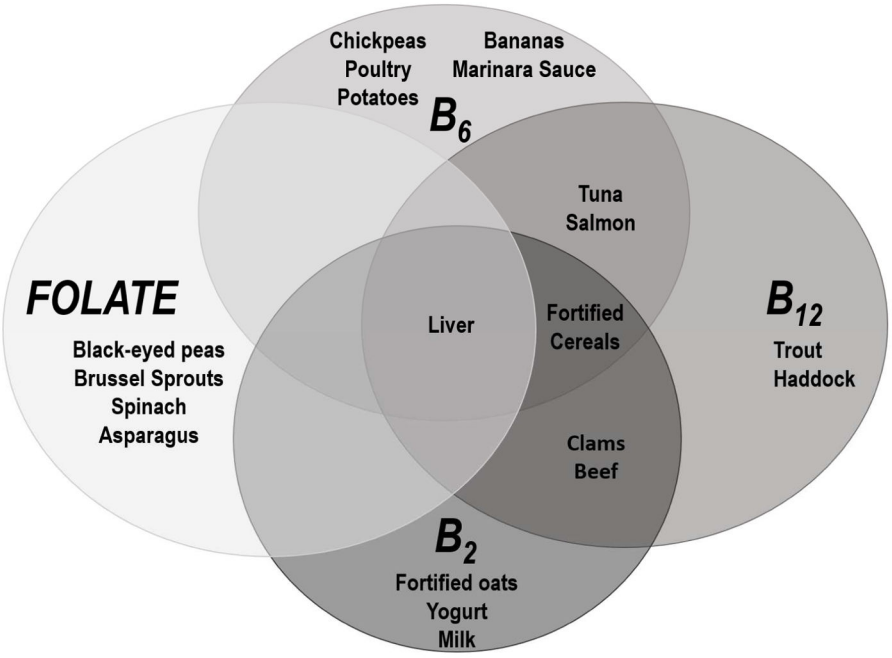

Figure 3. Major dietary sources of one-carbon metabolism (OCM) nutrients

Foods listed under OCM nutrients are considered to be high dietary sources of that nutrient (provide 20% or more of the daily value).(NIH 2016a) Foods fortified with folic acid are not included in the figure because U.S. folic acid fortification occurred after the SHS baseline visit.

Table 1

Participant Characteristics by Arsenic Metabolism (N=405)

	N	iAs% median (IQR)	P-value[a]	MMA% median (IQR)	P-value[a]	DMA% median (IQR)	P-value[a]
Overall	405	7.1 (5.2, 10.7)		13.7 (11.3, 17.7)		78.2 (71.6, 83.3)	
Age (years)							
<55	159	8.0 (5.8, 11.7)		13.3 (11.3, 18.0)		77.8 (70.6, 82.1)	
55 to <65	128	6.9 (5.3, 10.6)		13.5 (11.2, 17.5)		79.5 (71.0, 83.5)	
≥65	118	6.6 (4.4, 9.5)	0.007	14.6 (11.3, 17.4)	0.92	77.9 (73.1, 83.7)	0.46
Sex							
Male	181	9.2 (6.3, 12.7)		16.2 (13.0, 19.5)		75.1 (68.6, 80.2)	
Female	224	6.4 (4.6, 9.0)	<0.001	12.6 (9.6, 15.1)	<0.001	80.9 (75.7, 84.9)	<0.001
Σ As (μg/L)							
0.70 – <7.10	135	7.0 (5.0, 10.5)		13.8 (11.3, 17.2)		78.8 (73.1, 82.4)	
7.10 – 14.0	136	6.9 (4.6, 10.5)		14.2 (10.6, 17.9)		78.7 (71.7, 84.3)	
> 14.0 – 87.2	134	8.0 (5.6, 11.5)	0.07	13.3 (11.5, 18.2)	0.87	77.3 (70.4, 82.6)	0.23
BMI (kg/m^3)							
<25	72	10.3 (7.7, 12.4)		18.0 (13.2, 21.8)		71.6 (65.6, 77.3)	
25 to <30	141	7.1 (5.1, 10.6)		14.2 (11.8, 17.9)		77.9 (72.4, 82.5)	
≥30	192	6.5 (4.6, 9.7)	<0.001	12.8 (10.1, 15.9)	<0.001	80.6 (75.0, 84.7)	<0.001
Smoking Status							
Never	114	6.1 (4.3, 9.1)		12.8 (9.4, 15.6)		80.3 (75.0, 86.1)	
Former	138	6.8 (4.8, 9.9)		13.5 (11.7, 17.7)		79.1 (72.6, 83.7)	
Current	153	9.3 (6.4, 12.6)	<0.001	14.7 (12.3, 18.6)	<0.001	76.1 (68.8, 80.8)	<0.001
Alcohol							
Never	66	5.9 (4.0, 9.2)		12.8 (9.7, 15.9)		80.4 (73.6, 85.5)	
Former	188	6.9 (4.6, 10.3)		13.3 (10.5, 17.2)		79.4 (73.3, 84.0)	
Current	151	8.8 (6.4, 12.3)	<0.001	14.7 (12.6, 18.8)	<0.001	76.2 (68.8, 81.0)	<0.001
eGFR (mL/min)							
≤60	367	5.5 (3.8, 8.3)		13.3 (9.6, 16.8)		80.1 (75.1, 85.2)	
>60	38	7.5 (5.4, 10.9)	0.001	13.7 (11.3, 18.0)	0.55	78.1 (71.2, 82.7)	0.06
Urine Creatinine (mg/dL)							

	N	iAs% median (IQR)	P-value[a]	MMA% median (IQR)	P-value[a]	DMA% median (IQR)	P-value[a]
<0.95	136	8.5 (5.9, 11.9)		13.4 (11.4, 16.7)		78.0 (71.2, 81.9)	
0.95 – 1.50	134	6.4 (4.4, 9.3)		13.6 (10.5, 16.9)		79.4 (73.6, 85.0)	
>1.50	135	7.1 (5.0, 11.4)	<0.001	14.7 (11.9, 19.0)	0.12	78.2 (69.4, 83.1)	0.02
Total Caloric Intake (kcal)							
<1,230	135	7.5 (5.1, 10.5)		13.4 (11.3, 16.5)		78.7 (72.1, 83.3)	
1,230–1,968	135	8.1 (5.4, 11.9)		14.2 (10.8, 19.8)		77.0 (69.5, 83.4)	
>1,968	135	6.7 (5.0, 10.5)	0.14	13.7 (11.5, 17.3)	0.36	79.3 (72.5, 83.5)	0.24
Vitamin B_2 (mg)[b]							
<1.07	139	8.3 (5.4, 11.5)		14.1 (11.5, 18.8)		77.2 (69.9, 81.9)	
1.07–1.76	132	7.0 (4.9, 10.8)		13.3 (10.9, 17.8)		78.4 (72.0, 83.8)	
>1.76	134	6.9 (5.4, 10.1)	0.12	13.7 (10.8, 17.1)	0.39	79.0 (73.6, 83.5)	0.11
Vitamin B_6 (mg)[b]							
<0.93	135	8.5 (5.6, 12.9)		13.6 (11.5, 18.4)		76.9 (70.5, 82.1)	
0.93–1.68	135	7.7 (5.1, 11.4)		14.3 (11.4, 18.5)		77.8 (69.7, 82.6)	
>1.68	135	6.7 (4.9, 9.3)	0.005	13.2 (10.2, 16.9)	0.05	80.2 (74.8, 84.2)	0.005
Folate (μg)[b]							
<144.1	135	8.5 (5.7, 12.8)		14.1 (12.3, 18.0)		76.9 (69.9, 81.4)	
144.1–285.2	135	6.9 (4.9, 10.5)		13.2 (10.5, 17.7)		79.2 (72.1, 84.4)	
>285.2	135	6.9 (5.0, 10.2)	0.03	13.7 (10.0, 17.9)	0.22	79.3 (71.8, 83.7)	0.04
Vitamin B_{12} (μg)[b]							
<2.20	136	8.0 (5.3, 11.5)		14.2 (11.2, 18.7)		77.3 (70.7, 82.2)	
2.20–4.41	134	6.8 (4.9, 10.3)		13.3 (11.2, 17.1)		79.5 (72.6, 83.6)	
>4.41	135	7.2 (5.3, 11.2)	0.15	13.9 (11.3, 17.7)	0.53	78..2 (71.3, 83.5)	0.21

ΣAs, total urinary inorganic arsenic; BMI, body mass index; eGFR, estimated glomerular filtration rate

[a]Kruskal-Wallis tests were used to compare methylation medians across variable categories

[b]Vitamins are displayed as crude values, not calorie-adjusted

Table 2

Summary of Principal Components for Arsenic Species and Calorie-Adjusted Nutrition Variables

	As Metabolism PC1	As Metabolism PC2	OCM PC1	OCM PC3	OCM PC3	OCM PC4
Variance explained (%)	84.6	15.4	60.1	22.7	10.3	6.9
Standard deviation	1.59	0.68	1.55	0.95	0.64	0.52
Weights for iAs%	−0.55	−0.73	--	--	--	--
Weight for MMA%	−0.56	0.69	--	--	--	--
Weight for DMA%	0.63	−0.03	--	--	--	--
Weight for Vitamin B_2	--	--	0.57	0.13	0.47	−0.66
Weight for Vitamin B_6	--	--	0.53	−0.26	−0.79	−0.16
Weight for Folate	--	--	0.52	−0.46	0.37	0.62
Weight for Vitamin B_{12}	--	--	0.36	0.84	−0.12	0.39

As, arsenic; PC, principal component; OCM, one-carbon metabolism

Table 3

Mean Difference (95% CI) in iAs%, MMA% and DMA% by Calorie-Adjusted Nutrition Tertiles (N=405)

Model	Model 1[a]	Model 2[b]	Model 3[c]
Vitamin B_2 (mg)			
		iAs%	
<1.073	0.00 (ref)	0.00 (ref)	0.00 (ref)
1.073 – 1.43	−0.09 (−1.19, 1.01)	−0.21 (−1.20, 0.79)	−0.29 (−1.35, 0.77)
>1.43	−0.90 (−2.00, 0.20)	−1.00 (−2.00, 0.00)	−0.68 (−1.89, 0.53)
		MMA%	
<1.073	0.00 (ref)	0.00 (ref)	0.00 (ref)
1.073 – 1.43	−0.47 (−1.75, 0.81)	−0.59 (−1.71, 0.53)	−0.69 (−1.88, 0.50)
>1.43	−0.96 (−2.24, 0.33)	−1.36 (−2.48, −0.23)	−1.58 (−2.94, −0.22)
		DMA%	
<1.073	0.00 (ref)	0.00 (ref)	0.00 (ref)
1.073 – 1.43	0.57 (−1.53, 2.67)	0.80 (−1.01, 2.61)	0.98 (−0.99, 2.90)
>1.43	1.86 (−0.25, 3.97)	2.36 (0.54, 4.18)	2.26 (0.06, 4.46)
Vitamin B_6 (mg)			
		iAs%	
<0.936	0.00 (ref)	0.00 (ref)	0.00 (ref)
0.936 – 1.4241	−0.21 (−1.29, 0.88)	0.08 (−0.90, 1.06)	0.11 (−0.69, 1.39)
>1.4241	−1.92 (−3.01, −0.84)	−1.36 (−2.35, −0.37)	−1.18 (−2.32, −0.03)
		MMA%	
<0.936	0.00 (ref)	0.00 (ref)	0.00 (ref)
0.936 – 1.4241	−1.28 (−2.55, −0.02)	−0.78 (−1.89, 0.34)	−0.85 (−2.00, 0.30)
>1.4241	−2.29 (−3.56, −1.03)	−1.57 (−2.69, −0.45)	−1.80 (−3.09, −0.51)
		DMA%	
<0.936	0.00 (ref)	0.00 (ref)	0.00 (ref)
0.936 – 1.4241	1.49 (−0.58, 3.56)	0.69 (−1.10, 2.49)	0.74 (−1.12, 2.60)
>1.4241	4.21 (2.14, 6.28)	2.93 (1.13, 4.74)	2.98 (0.89, 5.07)
Folate (μg)			
		iAs%	
<149	0.00 (ref)	0.00 (ref)	0.00 (ref)
149 – 266	−0.67 (−1.77, 0.42)	−0.40 (−1.40, 0.60)	−0.06 (−1.10, 0.98)
>266	−1.31 (−2.41, −0.21)	−0.80 (−1.79, 0.20)	0.14 (−1.07, 1.34)
		MMA%	
<149	0.00 (ref)	0.00 (ref)	0.00 (ref)
149 – 266	−1.50 (−2.82, −0.27)	−0.93 (−2.06, 0.20)	−0.29 (−1.46, 0.88)
>266	−0.70 (−1.97, 0.58)	−0.18 (−1.30, 0.93)	1.27 (−0.08, 2.62)
		DMA%	

Model	Model 1[a]	Model 2[b]	Model 3[c]
<149	0.00 (ref)	0.00 (ref)	0.00 (ref)
149 – 266	2.22 (0.13, 4.32)	1.33 (−0.50, 3.16)	0.34 (−1.55, 2.23)
>266	2.01 (−0.09, 4.10)	0.98 (−0.83, 2.79)	−1.41 (−3.59, 0.78)
Vitamin B_{12} (μg)			
		iAs%	
<2.20	0.00 (ref)	0.00 (ref)	0.00 (ref)
2.20 – 3.93	0.36 (−0.74, 1.46)	0.52 (−0.47, 1.51)	0.74 (−0.31, 1.78)
>3.93	−0.27 (−1.37, 0.83)	−0.55 (−1.54, 0.44)	−0.06 (−1.16, 1.04)
		MMA%	
<2.20	0.00 (ref)	0.00 (ref)	0.00 (ref)
2.20 – 3.93	−0.51 (−1.80, 0.77)	−0.44 (−1.57, 0.68)	0.15 (−1.03, 1.32)
>3.93	0.01 (−1.28, 1.29)	−0.37 (−1.49, 0.76)	0.54 (−0.69, 1.78)
		DMA%	
<2.20	0.00 (ref)	0.00 (ref)	0.00 (ref)
2.20 – 3.93	0.15 (−1.96, 2.26)	−0.08 (−1.90, 1.74)	−0.89 (−2.79, 1.01)
>3.93	0.26 (−1.85, 2.38)	0.92 (−0.90, 2.74)	−0.48 (−2.48, 1.51)

[a]Adjusted for total log arsenic and log urinary creatinine

[b]Adjusted for total log arsenic, log urinary creatinine age, sex, center, smoking, alcohol, eGFR, BMI

[c]Adjusted for total log arsenic, log urinary creatinine age, sex, center, smoking, alcohol, eGFR, BMI, all b-vitamins of interest

Table 4

Mean Difference[a] (95% CI) in iAs%, MMA%, DMA% and Arsenic Principal Components by Nutrition Tertiles and OCM Principal Components

	As Metabolism PC1	As Metabolism PC2	iAs%	MMA%	DMA%
Calorie-Corrected Nutrition Tertiles					
Vitamin B_2 (mg)					
<1.073	0.00 (ref)	0.00 (ref)	0.00 (ref)	0.00 (ref)	0.00 (ref)
1.073 – 1.43	0.14 (−0.18, 0.47)	−0.05 (−0.20, 0.11)	−0.21 (−1.20, 0.79)	−0.59 (−1.71, 0.53)	0.80 (−1.01, 2.61)
>1.43	0.43 (0.10, 0.75)	−0.03 (−0.18, 0.13)	−1.00 (−2.00, 0.00)	−1.36 (−2.48, −0.23)	2.36 (0.54, 4.18)
Vitamin B_6 (mg)					
<0.936	0.00 (ref)	0.00 (ref)	0.00 (ref)	0.00 (ref)	0.00 (ref)
0.936 – 1.4241	0.12 (−0.20, 0.45)	−0.11 (−0.27, 0.04)	0.08 (−0.90, 1.06)	−0.78 (−1.89, 0.34)	0.69 (−1.10, 2.49)
>1.4241	0.53 (0.20, 0.86)	0.00 (−0.15, 0.16)	−1.36 (−2.35, −0.37)	−1.57 (−2.69, −0.45)	2.93 (1.13, 4.74)
Folate (μg)					
<149	0.00 (ref)	0.00 (ref)	0.00 (ref)	0.00 (ref)	0.00 (ref)
149 – 266	0.24 (−0.09, 0.57)	−0.06 (−0.22, 0.09)	−0.40 (−1.40, 0.60)	−0.93 (−2.06, 0.20)	1.33 (−0.50, 3.16)
>266	0.18 (−0.15, 0.51)	0.10 (−0.06, 0.25)	−0.80 (−1.79, 0.20)	−0.18 (−1.30, 0.93)	0.98 (−0.83, 2.79)
Vitamin B_{12} (μg)					
<2.20	0.00 (ref)	0.00 (ref)	0.00 (ref)	0.00 (ref)	0.00 (ref)
2.20 – 3.93	−0.02 (−0.35, 0.31)	−0.14 (−0.29, 0.02)	0.52 (−0.47, 1.51)	−0.44 (−1.57, 0.68)	−0.08 (−1.90, 1.74)
>3.93	0.17 (−0.16, 0.50)	0.04 (−0.12, 0.19)	−0.55 (−1.54, 0.44)	−0.37 (−1.49, 0.76)	0.92 (−0.90, 2.74)
One-Carbon Metabolism Principal Components					
OCM PC1	0.13 (0.04, 0.21)	0.00 (−0.04, 0.04)	−0.33 (−0.59, −0.07)	−0.38 − (0.67, −0.08)	0.71 (0.23, 1.18)
OCM PC2	0.03 (−0.11, 0.17)	−0.02 (−0.09, 0.04)	0.00 (−0.43, 0.43)	−0.17 (−0.65, 0.32)	0.17 (−0.62, 0.95)
OCM PC3	−0.14 (−0.35, 0.07)	0.02 (−0.08, 0.12)	0.29 (−0.34, 0.92)	0.48 (−0.23, 1.19)	−0.77 (−1.91, 0.38)
OCM PC4	−0.21 (−0.46, 0.05)	0.07 (−0.04, 0.20)	0.27 (−0.51, 1.05)	0.89 (0.01, 1.77)	−1.16 (−2.58, 0.27)

As, arsenic; PC, principal component; OCM, one-carbon metabolism

[a]Results adjusted for total log arsenic, log urinary creatinine, age, sex, center, smoking, alcohol, eGFR, BMI