Nutrients in one-carbon metabolism and urinary arsenic methylation in the National Health and Nutrition Examination Survey (NHANES) 2003-2004

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ABSTRACT

Exposure to inorganic arsenic (inAs), a potent toxicant, occurs primarily through ingestion of food and water. The efficiency with which it is methylated to mono and dimethyl arsenicals (MMA and DMA) affects toxicity. Folate, vitamins B12 and B6 are required for 1C metabolism, and studies have found that higher levels of these nutrients increase methylation capacity and are associated with protection against adverse health effects from inAs, especially in undernourished populations. Our aim was to determine whether 1C-related nutrients are associated with greater inAs methylation capacity in a general population sample with overall adequate nutrition and low levels of As exposure. Univariate and multivariable regression models were used to evaluate the relationship of dietary and blood nutrients to urinary As methylation in the National Health and Nutrition Examination Survey (NHANES) 2003-2004. Outcome variables were the percent of the sum of inAs and methylated As species (inAs + MMA + DMA) excreted as inAs, MMA, and DMA, and the ratio of MMA:DMA. In univariate models, dietary folate, vitamin B6 and protein intake were associated with lower urinary inAs% and greater DMA% in adults (≥18 years), with similar trends in children (6-18). In adjusted models, vitamin B6 intake (p=0.011) and RBC folate (p=0.036) were associated with lower inAs%, while dietary vitamin B12 was associated with higher inAs% (p=0.002) and lower DMA% (p=0.030). Total plasma homocysteine was associated with higher MMA% (p=0.004) and lower DMA% (p=0.003), but not with inAs%; other blood nutrients showed no association with urinary As. Although effect size is small, these findings suggest that 1C nutrients can influence inAs methylation and potentially play an indirect role in reducing toxicity in a general population sample.

INTRODUCTION

Arsenic (As) is a highly toxic element that occurs naturally in rock, soil and water, predominantly in inorganic form as arsenite (As(III)) and arsenate (As(V)). The majority of human exposure occurs via ingestion of As-contaminated water and food, but other sources of exposure include agricultural chemicals, mining and smelting of metal ores, coal-burning, wood preservatives and pharmaceuticals (IARC 2012). In the U.S., food is generally the primary source of As exposure (Xue et al. 2010; Kurzius-Spencer et al. 2014; Yager et al. 2015).

Arsenic occurs in food as both organic and inorganic compounds and toxicity depends on molecular form (Feldmann and Krupp 2011), oxidative state (+3 vs. +5) and on the extent of methylation (Sattar et al. 2016). Organic As, typically in the form of arsenobetaine (AsB), is found primarily in fish and other seafood and is generally assumed to be non-toxic. Inorganic As (inAs), found in a wide variety of foods including rice and rice products, other grains, fruit juices, vegetables and other foods, is considered highly toxic and has been linked to cancer (Hughes et al. 2011), cardiovascular disease (Tsuji et al. 2014), immune dysfunction (Farzan et al. 2016), diabetes (Wang et al. 2014) and altered neurodevelopment (Rodrigues et al. 2016). Inorganic As is metabolized in the liver via one-carbon (1C) metabolism, a biochemical pathway that is dependent on folate for the production of S-adenosylmethionine (SAM) (a universal methyl donor), and vitamin B12 (cobalamin) as a cofactor in the re-methylation of homocysteine to generate methionine (Gamble et al. 2005) (Figure 1). Metabolism of inAs involves a series of reduction/oxidation reactions that utilize glutathione (GSH) as the reducing agent, and a series of methylation steps utilizing SAM as the methyl donor. When As(V) is absorbed it is rapidly reduced to As(III). The first methylation step results in the formation of monomethylarsonic acid (MMA(V)), some of which may be reduced further, to form MMA(III). MMA undergoes a second

50 methylation to dimethylarsinic acid (DMA(V)), which may undergo further reduction to DMA(III).
51 Other nutrients associated with the 1C pathway include protein, as a source of amino acids
52 (methionine, cysteine, serine, glycine), and additional B vitamins, including B6 (Kile and
53 Ronnenberg 2008; Locasale 2013; Nijhout et al. 2008; Vahter 2002).
54
55 < FIGURE 1 >
56
57 While total urinary As includes all As species, organic and inorganic, the sum of inAs and
58 methylated As species excreted in urine (sumAs = As(III) + As(V) + MMA + DMA) is a frequently
59 used biomarker of inAs exposure (Kalman et al. 1990). The proportion of each As species
60 excreted in urine varies across individuals and reflects differences in exposure and As
61 methylation capacity (Kile and Ronnenberg 2008). The role of methylation in inAs detoxification
62 is somewhat uncertain given that some of the trivalent intermediate methylation products show
63 even greater toxicity than inAs (Agusa et al. 2011; Styblo et al. 2000; Vahter et al. 2007;
64 Valenzuela et al. 2005). Methylation, however, increases the rate of whole body clearance of
65 inAs (Drobna et al. 2010). Approximately 60-80% of inAs ingested is excreted in the form of
66 DMA(III+V), 10–30% as inAs (As(III+V)), and 2-30% as MMA(III+V) (Loffredo et al. 2003;
67 Schlebusch et al. 2015; Vahter 2000). SumAs is used as the denominator in assessing
68 differences in excretion patterns. Higher proportions of inAs and/or MMA are associated with
69 increased As retention in tissues, lower excretion rates and increased susceptibility to toxicity
70 (Pu et al. 2007; Schlebusch et al. 2015; Steinmaus et al. 2006; Vahter 2000; Valenzuela et al.
71 2005). A higher proportion of DMA is associated with greater methylation efficiency and
72 reduced toxicity. Variability in these ratios among populations has been attributed to
73 heterogeneity in the As(III) methyl transferase (AS3MT) gene, diet, age, gender, body mass,

74 nutritional status, and other factors (Chung et al. 2009; Engstrom et al. 2011; Gomez-Rubio et
75 al. 2010; Hennig B 2012; Howe et al. 2014; Steinmaus et al. 2005b).

76

77 A number of epidemiological studies have shown evidence that dietary and/or blood levels of
78 macro and micronutrients involved in 1C metabolism may protect against the adverse health
79 effects of chronic exposure to inAs (Anetor et al. 2007; Heck et al. 2007; Huang et al. 2008;
80 Pierce et al. 2011). These relationships are most apparent in folate (Gamble et al. 2006; Kile
81 and Ronnenberg 2008), cobalamin (Howe et al. 2014) and protein-deficient populations
82 (Steinmaus et al. 2005a). The evidence, however, is inconsistent. Several studies report no
83 significant associations between these nutrients and As methylation or susceptibility to As-
84 induced disease (Chung et al. 2006). Further, disparate results are reported for different age
85 groups (Gamble et al. 2005), and interaction effects have been observed with co-exposure to
86 other, non-nutrient factors (Basu et al. 2011).

87

88 Due to increasing public health concerns regarding possible adverse health effects of exposure
89 to inAs from food and water, our aim was to evaluate whether 1C nutrient levels (e.g., methyl
90 donors) might have a potential role in mitigating the toxic effects of dietary exposure in a general
91 population sample. Using data from NHANES 2003-2004 participants, aged 6 years and older,
92 we assessed the relationship of dietary folate, vitamins B12 and B6, and red blood cell (RBC)
93 folate, serum folate, serum vitamin B12, plasma vitamin B6, and total plasma homocysteine
94 (tHcys) to inAs metabolism. The relation to inAs metabolism of dietary protein, as a source of
95 certain amino acids that provide 1C units to the pathway, and plasma methylmalonic acid, as a
96 functional marker of vitamin B12 status, were also examined.

97

98

METHODS

Study Population

NHANES 2003-04 used a complex, multistage survey design to create “unbiased” estimates that are representative of the U.S. Census civilian non-institutionalized population (Curtin et al. 2012). Written consent/assent was obtained prior to participation. Participants were asked to complete an in-person interview that includes demographic, socioeconomic, health and dietary questions, and an examination component that involves laboratory and physiological tests and medical and dental exams. The physical exam component was administered within a few weeks of the interviews at mobile examination centers (MECs) (CDC 2013). A randomly selected one-third subset of participants, aged 6 years and older, provided spot urine samples for total and speciated arsenic analysis (Caldwell et al. 2009), and only the subset of participants who had urinary As measures and who had completed the dietary interview (first day) were included in the analyses presented here (n=2420). Appropriate sampling weights for this subset were used to account for differential selection probability due to cluster design, oversampling of certain subgroups, survey non-response and post-stratification (Curtin et al. 2012). Information on sex, race/ethnicity and age came from the demographic questionnaire; body mass index (BMI), calculated from measured height and weight, was available from the examination data. The race/ethnicity categories included non-Hispanic white, Mexican American, other Hispanic, non-Hispanic black and other race/multi-racial. The Adult questionnaire on Cigarette/Tobacco Use and the Adult Recent Tobacco Use and Youth Cigarette/Tobacco Use questionnaire were used to ascertain current smoking status for participants aged 12 and older. Participants who reported either smoking every day or smoking some days and/or smoking in the last five days were considered current smokers. Participants

who reported no current smoking and those under 12 years of age were coded as current non-smokers.

Dietary intake data

The dietary interview (first day) involved recall of detailed information on foods and beverages consumed over the 24 hour (h) period (midnight to midnight) prior to the interview (CDC 2007b). Individual and mixed food items were assigned values (concentrations in ng/g) for total As and inAs based on data from a market basket survey of foods comprising approximately 90% of dietary inAs intake in the U.S. (Schoof et al. 1999). Dietary As intake (μg/day) was then estimated based on As concentration in the foods (μg/kg) times the amount (g) consumed, as recorded in the dietary interview. These methods have been described in detail elsewhere (Kurzius-Spencer et al. 2014). Total nutrient intake was included in the dietary interview data, calculated using the USDA Food and Nutrient Database for Dietary Studies (FNDDS) 2.0 (CDC 2007b). Consumption of seafood or rice was determined from the specific FNDDS 2.0 food codes for foods containing fish, shellfish and other seafood and for foods containing white, brown or wild rice, rice flour or rice cereal, respectively, as reported in the dietary interview.

Total energy (kilocalorie) intake was evaluated, based on expected energy expenditure (Mifflin-St. Jeor equations) for adults (Mifflin et al. 1990) and total energy expenditure equations for children (James et al. 1990). Dietary As and nutrient intake were adjusted for extreme under-reporting (<75%) and over-reporting (>150%) of expected energy intake, prior to modeling.

Urinary As

Spot urine samples were collected from participants ≥6 years of age at the mobile clinics. Inductively coupled plasma-dynamic reaction cell-mass spectrometry (ICP-DRC-MS) was used

to measure urinary total As (limit of detection = 0.74 µg/l) and high-performance liquid chromatography (HPLC) coupled to ICP-DRC-MS was used to measure urinary inorganic and organic As species. Details of these methods have been described elsewhere (Caldwell et al. 2009; CDC 2007a). Of the As species measured, As(V) and As(III) were below the limit of detection (LOD) in greater than 92 and 95% of samples, with LODs of 1.0 and 1.2 µg/l, respectively (see Table 1). The LOD for AsB, DMA, and MMA were 0.4, 1.7 and 0.9 µg/l, respectively. AsB and DMA were above the LOD in >60% of participants, while MMA was above the LOD in 35% (Caldwell et al. 2009). Urinary As outcome variables were defined as shown in Table 1. Urinary creatinine was measured in the samples using a CX3 Analyzer and a Jaffe rate reaction.

< TABLE 1 >

Blood nutrients

The laboratory methods for quantification of serum and red blood cell (RBC) folate and serum vitamin B12 concentrations in the 2003-2004 NHANES involved use of a commercially available radioprotein binding assay kit (Quantaphase II; BioRad Laboratories, Hercules, CA). Assessment of plasma pyridoxal 5'-phosphate (PLP), the biologically active form of vitamin B6, used the Enzymatic B6 Assay (A/C Diagnostics, San Diego, CA, USA). Total homocysteine (tHcys) concentration in plasma was measured by a fully automated fluorescence polarization immunoassay on the Abbott AxSym system (Abbott Laboratories, Abbott Park, IL), and assessment of plasma methylmalonic acid concentrations involved gas chromatography–mass spectrometry with cyclohexanol derivatization. Laboratory methods are described in detail online (CDC).

172 *Statistical analysis*

173 STATA/SE 11.2 was used for data analysis. The distribution of variables of interest were
174 evaluated for normality and the proportion of values below the LOD. Prior to modeling, right-
175 skewed variables were log(10)-transformed. Survey methods and sampling weights specific to
176 this population subsample were used to calculate weighted proportions for categorical variables,
177 geometric means (GM) and standard errors (SE) for log-transformed variables, and linearized
178 variance estimates in the regression models. Univariate, multivariable and nested regression
179 models were run.

180

181 The following urinary As biomarkers were the dependent variables in the models: inAs%,
182 MMA% (primary methylation index or PMI), DMA%, and DMA/MMA (secondary methylation
183 index or SMI). The covariates of interest in the models included dietary nutrient intake (total
184 folate, total protein, vitamin B6 and B12) and blood nutrient status (RBC folate, serum folate,
185 serum vitamin B12, plasma vitamin B6, plasma tHcys, and plasma methylmalonic acid). Sex,
186 age, race/ethnicity, urinary creatinine, BMI, current smoking status, total energy intake, dietary
187 As intake (total and inAs), seafood intake in past 24 h and rice intake in past 24 h were included
188 as potential confounders. Univariate models were run on the entire population and on the
189 population stratified by age group (children, age 6 to <18 years, and adults, age ≥18 years) to
190 determine whether the relation between nutrients and inAs metabolism differed in children and
191 adults. Marginal analyses to assess the relationship between urine creatinine and urinary As
192 biomarkers and urine creatinine with other covariates in the models were performed. Nested
193 multivariable regression models were built by sequentially adding blocks of related variables.
194 These blocks consisted of: 1) dietary As intake (dietary total As, dietary inAs, indicator variables
195 for seafood or rice consumption), 2) demographic and lifestyle confounders (i.e., race/ethnicity,
196 sex, age, BMI and current smoking), 3) urine creatinine, 4) dietary nutrient intake (total protein,

197 total folate, Vit B12, Vit B6), 5) blood nutrient concentrations (RBC folate, serum folate, serum
198 Vit B12, plasma Vit B6, plasma tHcys, plasma methylmalonic acid), 6) and interaction terms
199 (creatinine X sex, creatinine X BMI). The Wald test was used to test the significance of each
200 block of predictors. Change in R^2 and adjusted R^2 values are reported.
201
202
203 **RESULTS**
204
205 *Population characteristics and exposures*
206 Among the participants in NHANES 2003-04, a subsample of 2420 participants had valid data
207 for urinary As, dietary intake, and 1C-related biomarkers. The weighted population sample was
208 slightly more than 50% female, 71% non-Hispanic white, 11.7% non-Hispanic black, 8.7%
209 Mexican-American, and “other Hispanic” and “other race” comprising the remainder (8.7%)
210 (Table 2). Age ranged from 6-85 years, with a median age of 29 years. Approximately 22% of
211 the population were current smokers (not shown).
212
213 < TABLE 2 >
214
215 The GM intake of total and inorganic As from food was 41.08 ±1.03 and 6.20 ± 1.02 μg/day,
216 respectively. Dietary intake of total As was significantly higher among other Hispanics, non-
217 Hispanic blacks and other races, as compared to non-Hispanic whites, but did not differ
218 between Mexican-Americans and non-Hispanic whites. Dietary inAs intake was also
219 significantly higher among Mexican-Americans, other Hispanics and other races, as compared
220 to non-Hispanic whites. Percent inAs in the diet was significantly lower among non-Hispanic
221 blacks as compared with non-Hispanic whites, but other racial/ethnic groups were not

significantly different from non-Hispanic whites (not shown). A greater proportion of other Hispanics, non-Hispanic blacks and those of other race reported having consumed seafood and/or rice during the past 24 h, as compared with non-Hispanic whites and Mexican-Americans. Dietary intake of all of the nutrients was at or above the dietary reference intake values (National Academy of Sciences (US) 2011) for 95% of children 6-18 and for 50-75% of adults (≥18) and blood nutrients were within or above the reference ranges (CDC ; CDC.gov) for at least 75% of all participants (data not shown). Nutrient blood levels, which can indicate long-term nutritional status (Green 2011), were within the reference ranges in greater than 90% of the population, with the exception of vitamin B6, which was within the reference range for over 50% the population (CDC ; CDC.gov) (data not shown). Although there were few ethnic differences in nutrient intake, nutrient status varied among ethnic/racial groups. Non-Hispanic whites tended to have higher blood levels of folate (RBC and serum), vitamin B6, tHcys and methylmalonic acid, and lower serum levels of vitamin B12 than other ethnic/racial groups. The distribution of urinary As biomarkers also differed by race/ethnicity (Table 3). When compared with non-Hispanic whites, all other ethnic/racial groups had higher GM levels of urinary total As, sumAs and DMA%, and lower inAs% and MMA%.

< TABLE 3 >

In marginal analyses, urinary creatinine was significantly associated with all urinary As biomarkers and with sex, age, current smoking, race/ethnicity and BMI (not shown). Urine creatinine explained 39% of the variance in inAs%, 3% of MMA%, 32% of DMA%, and 15% of the ratio of DMA:MMA. 1C nutrient levels in blood were negatively associated with creatinine (all $p<0.10$) (not shown).

Univariate models

The crude relations between urinary As biomarkers and demographic, dietary As, dietary and blood nutrients and other covariates are presented in Table 4. As compared to males, females had higher urinary inAs% and lower DMA%, and inAs% was also associated with age. BMI was associated with a lower PMI and higher SMI, in contrast to current smoking, which was associated with a higher PMI and lower SMI. Race/ethnicity accounted for 3-7% of the variance in the urinary biomarkers (not shown).

< TABLE 4 >

All of the dietary As variables were inversely related to the PMI and inAs% and positively related to the SMI. Total As intake from food explained approximately 12% of the variance in DMA%, but only 5.5% of the PMI, while dietary inAs accounted for 6% of the variance in DMA% and 1% of the PMI. Seafood consumption in the previous 24 h explained 7% of the variance in DMA% and 4% of the PMI (both $p<0.001$), while rice consumption explained about 3% and 1%, respectively ($p=0.001$ and 0.004).

Dietary folate ($p=0.006$), vitamin B6 ($p=0.034$) and total protein ($p=0.005$) were each inversely associated with inAs% ($p=0.011$) in the crude models. Dietary folate was also associated with DMA% ($p=0.005$) while vitamin B6 and dietary protein showed positive trends with DMA%, though none of the individual nutrients explained more than 1% of the variance in biomarkers. In the crude analyses, there were no significant associations between blood nutrients and urinary As biomarkers. Analyses stratified by age group (children between 6 and 18 years old vs. adults ≥18.0 years old) demonstrated similar trends (Table 5), although none of the nutrients showed statistical significance in children only.

< TABLE 5 >

Multivariable models

In the nested multivariable regression models (Table 6), the first three blocks – dietary As intake, confounders, and urinary creatinine – explained the majority of variance. The change in R^2 with entry of blocks 1 through 3 was significant in all of the outcome models. Inclusion of Block 4, dietary nutrients, caused a small but statistically significant R^2 change in urinary inAs% (p=0.034) and DMA% (p=0.025). Blood nutrient status, block 5, was associated with MMA% (p=0.018), DMA% (p=0.043) and SMI (p=0.018), after adjustment. The significance of interaction effects varied by outcome. Interaction between creatinine and race/ethnic groups was not significant in these models and hence was not included.

In the full models, dietary intake of vitamin B6 was independently associated with lower inAs% (p=0.011), as was RBC folate (p=0.036). In contrast, dietary intake of vitamin B12 was associated with higher inAs% and lower DMA%. Higher plasma tHcys was associated with the PMI (p= 0.004), and inversely associated with the SMI (p=0.001) and DMA% (p=0.003).

< TABLE 6 >

To determine whether the models were sensitive to the specific variables included as controls for dietary As intake, sensitivity analyses were used to compare models with dietary inAs intake, with and without variables indicating seafood and/or rice consumption. Regardless of which dietary intake variable(s) was used, the relation of nutrient intake and nutrient status were consistent. Substitution of dietary inAs for total As yielded highly comparable adjusted R^2

297 values, but inclusion of seafood or rice consumption alone accounted for a smaller proportion of
298 the variance in the models.
299
300
301 **DISCUSSION**
302
303 This study shows an independent relationship between one-carbon/methyl donor nutrients and
304 urinary As methylation in a representative sample of the U.S. population (NHANES 2003-04),
305 with a very small overall effect size as measured by R^2. In models adjusted for age, sex,
306 race/ethnicity, current smoking, BMI, dietary As intake, urine creatinine and interaction factors,
307 dietary intake of vitamin B6 and red blood cell folate were associated with a lower inAs%, while
308 dietary vitamin B12 was associated with higher inAs%. An inverse relationship to secondary
309 methylation was observed for dietary vitamin B12 and plasma tHcys, and tHcys was also
310 directly related to a higher proportion of urinary As excreted after the primary methylation step.
311 Dietary folate and protein were associated with As metabolism only in crude models. Nutrients
312 in the blood, other than tHcys, showed no relation to As metabolism in either crude or adjusted
313 models.
314
315 Nutrients in the 1C pathway have previously been shown to facilitate As methylation and protect
316 against toxicity, especially in nutrient-deficient populations (Gamble et al. 2005; Gamble et al.
317 2006; Gamble et al. 2007; Hall et al. 2009b; Heck et al. 2007; Kordas et al. 2016; Zablotska et
318 al. 2008). Despite relatively low As exposure in the U.S. population and generally adequate
319 nutrition, our results corroborate those reported in several studies of highly As-exposed
320 populations with high rates of undernutrition. As in the present study, plasma tHcys was
321 positively associated with primary methylation and negatively associated with secondary

methylation among adults in As-endemic areas of Bangladesh (Gamble et al. 2005; Hall et al. 2007), suggesting possible interference with secondary methylation. Also, elevated tHcys tends to be associated with low levels of the other 1C-related micronutrients (Green 2011). Among children in Bangladesh, however, plasma folate levels were inversely related to urinary inAs% and higher tHcys was associated with lower primary methylation of As (statistically significant in males only). In the same study there was no relationship between plasma vitamin B12 and urinary As methylation (Hall et al. 2009b).

Importantly, in our study, higher RBC folate, a marker for long-term folate status (Green 2011), was associated with a reduction in excretion of unmethylated metabolites (inAs%) and showed a non-significant positive relationship with DMA%. A randomized controlled study involving folic acid supplementation in populations with low plasma folate demonstrated 10-15% reductions in the proportion of total urinary As excreted as inAs and MMA (Gamble et al. 2006) and a 22% reduction in MMA in blood (Gamble et al. 2007). Animal studies have also shown reductions in As-induced toxicity, due to oxidative stress (Ma et al. 2015; Majumdar et al. 2009) and DNA damage (Acharyya et al. 2015; Majumdar et al. 2009), with folic acid supplementation.

Conflicting results on the effect of vitamin B12 on As methylation have been reported, even in highly exposed populations. In a study of highly-exposed children in Bangladesh, plasma vitamin B12 was not associated with urinary As methylation (Hall et al. 2009b), however, B12 was positively related to primary methylation in several studies involving adults (Hall et al. 2009a; Howe et al. 2014). Howe et al. (2014) hypothesized a competition between inAs and MMA for methyl donors in subjects with chronic exposure to high levels of inAs (Howe et al. 2014). Null effects in some studies were attributed to the exclusion of vitamin B12-deficient participants or to adequate B12 status among participants (Zablotska et al. 2008). In the 2003-

347 04 NHANES population, we observed an increase in inAs% and a decrease in DMA%
348 associated with dietary intake, but no effect on primary methylation. Furthermore, we found no
349 effect of biomarkers of vitamin B12 – i.e., serum vitamin B12 or methylmalonic acid – on inAs
350 metabolism.
351
352 Most prior studies were conducted in populations with generally low dietary intake of protein and
353 it is unclear whether the participants had sufficient levels of methionine, choline and/or cysteine
354 to metabolize inAs to DMA (Steinmaus et al. 2005a). Higher intake of dietary protein has been
355 associated with lower urinary inAs% in Bangladesh (Heck et al. 2007; Heck et al. 2009) and
356 higher secondary methylation in an As-exposed population in the western U.S. (Steinmaus et al.
357 2005a). In the Steinmaus study, participants in the lowest quartile of protein, iron, thiamin,
358 niacin, vitamin B6, zinc, and α-carotene intake, as compared with those in the upper quartile,
359 excreted a higher proportion of MMA and a lower proportion of DMA, however, folate intake was
360 unrelated to As metabolism (Steinmaus et al. 2005a). In crude models only, we observed
361 strong inverse relationships between dietary protein and dietary folate and inAs%, and positive
362 associations with secondary methylation. The loss of statistical significance in the adjusted
363 models may be an effect of fairly high pairwise correlations (Spearman rho 0.42-0.57) between
364 dietary intake of protein and vitamins B12 and B6, and between dietary intake of total folate and
365 vitamins B12 and B6. While correlations between dietary and blood nutrients in our study were
366 statistically significant, the correlation coefficients were low (all rho <0.15).
367
368 The nutrients and biomarkers selected for this analysis were those commonly studied in relation
369 to 1C metabolism. Information on AS3MT genotype and on additional analytes, including SAM,
370 were not available in the NHANES 2003-04 dataset, but could have provided more insight into
371 individual differences in metabolism. Howe et al. (2014) found that SAM was not correlated with

plasma folate, and that increased concentrations of SAM inhibited production of 5-methyl tetrahydrofolate, the biologically active form of folate. Vitamin B12, on the other hand, was positively correlated with SAM, and SAM concentrations were higher in participants with sufficient B12 levels (Howe et al. 2014).

There is ongoing debate regarding adjustment of urinary As values for creatinine to correct for variable water excretion rates. The synthesis of creatinine, like the metabolism of inAs, requires SAM derived from 1C metabolism and is closely associated with urinary As methylation (Gamble and Liu 2005; Nermell et al. 2008), 1C nutrients (Basu et al. 2011), and most of the other covariates included in our models. According to Brosnan et al (2011), 40% of SAM-derived methyl groups are involved in the synthesis of creatine, hence, when creatine levels are low, availability of SAM for As methylation may be limited (Brosnan et al. 2011). Because of the complexity of its relationships with the covariates in our models, urinary As biomarkers were not adjusted for creatinine. Instead, as argued by others (Basu et al. 2011; Gamble and Hall 2012), creatinine was included as a confounder and potential interaction effects with sex, race/ethnicity, smoking and BMI were tested in the models.

Although environmental As exposure was not measured in NHANES 2003-04, we made the assumption, based on average exposures in the U.S. (ATSDR 2007), that exposure from sources other than food was uniform and relatively low. In a study that modeled exposure to inAs in three U.S. study populations (including NHANES 2003-04), we estimated that the geometric mean aggregate inAs exposure was 9-12 µg/day among participants with tap water As concentrations <10 ppb, and that 54 - 75% of intake was from food (Kurzius-Spencer et al. 2014). Our estimates of dietary intake of inAs were based on a single dietary recall record and a 1999 market basket survey of inAs in foods that were thought to comprise approximately 90%

of intake (Schoof et al. 1999). Recognizing that reporting bias is inherent in dietary recalls and other indirect methods of assessing As in the food, we nevertheless found that modeled dietary As intake was a better predictor of urinary As than was As exposure from water (Kurzius-Spencer et al. 2013).

Data on dietary intake of DMA and As species other than inAs (and on the metabolism and toxicity of methylated As species) is generally lacking (deCastro et al. 2014). In evaluating urinary DMA and MMA, we are unable to differentiate between exogenous exposure to methylated arsenicals from foods consumed and endogenous exposure via metabolism of inAs. Urinary DMA has been associated with consumption of fish (Aylward et al. 2014), rice and rice products (deCastro et al. 2014), and urinary MMA primarily with rice and rice products (with several other food groups showing marginal effects) (deCastro et al. 2014). The positive relationship we observed between dietary As (and consumption of seafood or rice) and urinary DMA% (Table 4) and between 1-C nutrients and DMA% (Table 5) could be attributable to consumption of folic acid-enriched foods (including rice) that are also a source of exposure to DMA. If, as cited above, rice consumption is associated with urinary MMA (deCastro et al. 2014), the statistically significant inverse relationships observed in our adjusted models (Table 6) between rice consumption and urinary MMA% and inAs%, and the positive relationship with DMA%, suggest an increase in secondary methylation efficiency.

A number of previous studies have reported on aspects of diet and urinary As speciation in NHANES populations (deCastro et al. 2014; Kurzius-Spencer et al. 2013; Navas-Acien et al. 2011; Xue et al. 2010). Data from NHANES 2003-2006 showed that seafood consumption in the previous 24 h was the major determinant, not just of urinary total As and arsenobetaine, but also of DMA. Yet, select sources of As, such as seafood, may not be habitually consumed on a

daily basis. Thus, a single day of intake is likely insufficient to capture true dietary exposure. Further, while generally considered a marker of inAs exposure, urinary DMA may also result from direct exposure to a wide variety of plant and animal-based foods (deCastro et al. 2014) and potentially from metabolism of organoarsenicals in seafoods (Aylward et al. 2014). Consumption or rice and rice products is another important potential contributor to urinary As concentrations (Karagas et al. 2016; Wu et al. 2015). Hence, both seafood consumption and rice consumption during the previous 24 h were included as covariates in our models.

Although these analyses included a representative population sample, differences in nutrient metabolism and the metabolism of inAs among children, adults, women of child-bearing age, and different ethnic/racial groups have been reported. We attempted to address some of these differences by including age, sex and ethnicity as confounders in our models and by running sensitivity analyses, however, it is quite possible that our findings are affected by residual confounding.

Dietary supplement use was not included in these analyses. Due to the very large number of dietary supplements available, an in-depth assessment of supplement utilization would involve evaluation of combinations of nutrients, variable doses, and miscellaneous ingredients contained in each supplement, which was beyond the scope of this paper.

A concern regarding analysis of urinary As metabolites in a population with relatively low As exposure is the high proportion of As values below the limit of detection -- over 90% of As(III) and As(V) and 65% of MMA concentrations. The effect on the urinary As outcome biomarkers is presumed to be non-differential and to bias the results toward the null hypothesis.

Strengths of the analysis included a well-characterized and relatively large, nationally representative sample of individuals and the evaluation of diet and several blood nutrients in relation to urinary As exposure.

CONCLUSION

Our findings have important implications related to inAs exposure in the U.S. Higher intake and metabolic levels of folate and vitamin B6 appear to mitigate inAs toxicity even in a population with relatively low As exposure and a generally nutrient-sufficient diet. At the same time, higher total plasma homocysteine levels and higher dietary intake of vitamin B12 may interfere with As methylation and result in a greater susceptibility to inAs exposure. Controlled intervention studies are needed to more robustly assess the feasibility and efficacy of modifying nutrient intake in order to enhance As methylation capacity and, in turn, reduce As toxicity in the general population.

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REFERENCES

Acharyya N, Deb B, Chattopadhyay S, Maiti S. 2015. Arsenic-induced antioxidant depletion, oxidative DNA breakage, and tissue damages are prevented by the combined action of folate and vitamin b12. Biological Trace Element Research 168:122-132.

Agusa T, Fujihara J, Takeshita H, Iwata H. 2011. Individual variations in inorganic arsenic metabolism associated with as3mt genetic polymorphisms. Int J Mol Sci 12:2351-2382.

Anetor JI, Wanibuchi H, Fukushima S. 2007. Arsenic exposure and its health effects and risk of cancer in developing countries: Micronutrients as host defence. Asian Pac J Cancer Prev 8:13-23.

ATSDR. 2007. Agency for toxic substances and disease registry. Toxicological profile for arsenic (update). Available: http://www.atsdr.cdc.gov/toxprofiles/tp2.pdf [accessed 3/23/17].

Aylward LL, Ramasamy S, Hays SM, Schoeny R, Kirman CR. 2014. Evaluation of urinary speciated arsenic in nhanes: Issues in interpretation in the context of potential inorganic arsenic exposure. Regulatory Toxicology And Pharmacology : RTP 69:49-54.

Basu A, Mitra S, Chung J, Guha Mazumder DN, Ghosh N, Kalman D, et al. 2011. Creatinine, diet, micronutrients, and arsenic methylation in west bengal, india. Environmental Health Perspectives 119:1308-1313.

Brosnan JT, da Silva RP, Brosnan ME. 2011. The metabolic burden of creatine synthesis. Amino Acids 40:1325-1331.

Caldwell KL, Jones RL, Verdon CP, Jarrett JM, Caudill SP, Osterloh JD. 2009. Levels of urinary total and speciated arsenic in the US population: National Health and Nutrition Examination Survey 2003-2004. J Expo Sci Environ Epidemiol 19:59-68.

CDC. Nhanes 2003-2004 lab methods. Available: https://www.cdc.gov/nchs/nhanes/nhanes2003-2004/lab_methods_03_04.htm [accessed December 16, 2016.

CDC. National health and nutrition examination survey, nhanes 2003-2004. Available: https://wwwn.cdc.gov/nchs/nhanes/Search/nhanes03_04.aspx [accessed Dec 16, 2016].

CDC. 2007a. National health and nutrition examination survey, 2003-2004 data documentation, codebook, and frequencies. Arsenics - total and speciated - urine. Available: https://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/L06UAS_C.htm [accessed Nov 30 2008].

CDC. 2007b. National health and nutrition survey 2003-2004 data documentation, codebook and frequencies, dietary interview - individual foods, first day (dr1iff_c). Available: https://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/DR1IFF_C.htm 2008].

CDC. 2013. Sample design. Available: https://www.cdc.gov/nchs/tutorials/Nhanes/SurveyDesign/SampleDesign/intro.htm [accessed May 10, 2017.

Chung CJ, Hsueh YM, Bai CH, Huang YK, Huang YL, Yang MH, et al. 2009. Polymorphisms in arsenic metabolism genes, urinary arsenic methylation profile and cancer. Cancer Causes Control 20:1653-1661.

Chung JS, Haque R, Guha Mazumder DN, Moore LE, Ghosh N, Samanta S, et al. 2006. Blood concentrations of methionine, selenium, beta-carotene, and other micronutrients in a case-control study of arsenic-induced skin lesions in West Bengal, India. Environmental Research 101:230-237.

Curtin LR, Mohadjer LK, Dohrmann SM, Montaquila JM, Kruszan-Moran D, Mirel LB, et al. 2012. The National Health and Nutrition Examination Survey: Sample design, 1999-2006. Vital Health Stat 2:1-39.

deCastro BR, Caldwell KL, Jones RL, Blount BC, Pan Y, Ward C, et al. 2014. Dietary sources of methylated arsenic species in urine of the united states population, NHANES 2003-2010. PloS One 9:e108098.

Drobna Z, Walton FS, Harmon AW, Thomas DJ, Styblo M. 2010. Interspecies differences in metabolism of arsenic by cultured primary hepatocytes. Toxicology and Applied Pharmacology 245:47-56.

Engstrom K, Vahter M, Mlakar SJ, Concha G, Nermell B, Raqib R, et al. 2011. Polymorphisms in arsenic(+iii oxidation state) methyltransferase (as3mt) predict gene expression of as3mt as well as arsenic metabolism. Environmental Health Perspectives 119:182-188.

Farzan SF, Li Z, Korrick SA, Spiegelman D, Enelow R, Nadeau K, et al. 2016. Infant infections and respiratory symptoms in relation to in utero arsenic exposure in a u.S. Cohort. Environmental Health Perspectives 124:840-847.

Feldmann J, Krupp EM. 2011. Critical review or scientific opinion paper: Arsenosugars--a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs? Anal Bioanal Chem 399:1735-1741.

Gamble MV, Liu X. 2005. Urinary creatinine and arsenic metabolism. Environmental Health Perspectives 113:A442; author reply A442-443.

Gamble MV, Liu X, Ahsan H, Pilsner R, Ilievski V, Slavkovich V, et al. 2005. Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. Environmental Health Perspectives 113:1683-1688.

Gamble MV, Liu X, Ahsan H, Pilsner JR, Ilievski V, Slavkovich V, et al. 2006. Folate and arsenic metabolism: A double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. Am J Clin Nutr 84:1093-1101.

Gamble MV, Liu X, Slavkovich V, Pilsner JR, Ilievski V, Factor-Litvak P, et al. 2007. Folic acid supplementation lowers blood arsenic. Am J Clin Nutr 86:1202-1209.

Gamble MV, Hall MN. 2012. Relationship of creatinine and nutrition with arsenic metabolism. Environmental Health Perspectives 120:A145-146.

Gomez-Rubio P, Meza-Montenegro MM, Cantu-Soto E, Klimecki WT. 2010. Genetic association between intronic variants in as3mt and arsenic methylation efficiency is focused on a large linkage disequilibrium cluster in chromosome 10. J Appl Toxicol 30:260-270.

Green R. 2011. Indicators for assessing folate and vitamin b-12 status and for monitoring the efficacy of intervention strategies. Am J Clin Nutr 94(suppl):666S-672S.

Hall M, Gamble M, Slavkovich V, Liu X, Levy D, Cheng Z, et al. 2007. Determinants of arsenic metabolism: Blood arsenic metabolites, plasma folate, cobalamin, and homocysteine concentrations in maternal-newborn pairs. Environmental Health Perspectives 115:1503-1509.

Hall MN, Liu X, Slavkovich V, Ilievski V, Mi Z, Alam S, et al. 2009a. Influence of cobalamin on arsenic metabolism in Bangladesh. Environmental Health Perspectives 117:1724-1729.

Hall MN, Liu X, Slavkovich V, Ilievski V, Pilsner JR, Alam S, et al. 2009b. Folate, cobalamin, cysteine, homocysteine, and arsenic metabolism among children in Bangladesh. Environmental Health Perspectives 117:825-831.

Heck JE, Gamble MV, Chen Y, Graziano JH, Slavkovich V, Parvez F, et al. 2007. Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh. Am J Clin Nutr 85:1367-1374.

Heck JE, Nieves JW, Chen Y, Parvez F, Brandt-Rauf PW, Graziano JH, et al. 2009. Dietary intake of methionine, cysteine, and protein and urinary arsenic excretion in Bangladesh. Environmental Health Perspectives 117:99-104.

Hennig B OL, McClain CJ, Watkins BA, Blumberg B, Bachas LG, Sanderson W, Thompson C, Suk WA. 2012. Nutrition can modulate the toxicity of environmental pollutants: Implications in risk assessment and human health. Environmental Health Perspectives 120:771-774.

Howe CG, Niedzwiecki MM, Hall MN, Liu X, Ilievski V, Slavkovich V, et al. 2014. Folate and cobalamin modify associations between s-adenosylmethionine and methylated arsenic metabolites in arsenic-exposed Bangladeshi adults. J Nutr 144:690-697.

Huang YK, Pu YS, Chung CJ, Shiue HS, Yang MH, Chen CJ, et al. 2008. Plasma folate level, urinary arsenic methylation profiles, and urothelial carcinoma susceptibility. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 46:929-938.

Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ. 2011. Arsenic exposure and toxicology: A historical perspective. Toxicological sciences : an official journal of the Society of Toxicology 123:305-332.

IARC. 2012. A review of human carcinogens: Arsenic, metals, fibres, and dusts.

James WPT, Schofield C, Food and Agriculture Organization of the United Nations. 1990. Human energy requirements : A manual for planners and nutritionists. Oxford ; New York:Published by arrangement with the Food and Agriculture Organization of the United Nations by Oxford University Press.

Kalman DA, Hughes J, van Belle G, Burbacher T, Bolgiano D, Coble K, et al. 1990. The effect of variable environmental arsenic contamination on urinary concentrations of arsenic species. Environmental Health Perspectives 89:145-151.

Karagas MR, Punshon T, Sayarath V, Jackson BP, Folt CL, Cottingham KL. 2016. Association of rice and rice-product consumption with arsenic exposure early in life. JAMA pediatrics 170:609-616.

Kile ML, Ronnenberg AG. 2008. Can folate intake reduce arsenic toxicity? Nutr Rev 66:349-353.

Kordas K, Queirolo EI, Manay N, Peregalli F, Hsiao PY, Lu Y, et al. 2016. Low-level arsenic exposure: Nutritional and dietary predictors in first-grade Uruguayan children. Environmental Research 147:16-23.

Kurzius-Spencer M, O'Rourke MK, Hsu CH, Hartz V, Harris RB, Burgess JL. 2013. Measured versus modeled dietary arsenic and relation to urinary arsenic excretion and total exposure. J Expo Sci Environ Epidemiol 23:442-449.

Kurzius-Spencer M, Burgess JL, Harris RB, Hartz V, Roberge J, Huang S, et al. 2014. Contribution of diet to aggregate arsenic exposures-an analysis across populations. J Expo Sci Environ Epidemiol 24:156-162.

Locasale JW. 2013. Serine, glycine and the one-carbon cycle: Cancer metabolism in full circle. Nat Rev Cancer 13:572-583.

Loffredo CA, Aposhian HV, Cebrian ME, Yamauchi H, Silbergeld EK. 2003. Variability in human metabolism of arsenic. Environmental Research 92:85-91.

Ma Y, Zhang C, Gao XB, Luo HY, Chen Y, Li HH, et al. 2015. Folic acid protects against arsenic-mediated embryo toxicity by up-regulating the expression of Dvr1. Sci Rep 5:16093.

Majumdar S, Mukherjee S, Maiti A, Karmakar S, Das AS, Mukherjee M, et al. 2009. Folic acid or combination of folic acid and vitamin b(12) prevents short-term arsenic trioxide-induced systemic and mitochondrial dysfunction and DNA damage. Environmental Toxicology 24:377-387.

Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. 1990. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 51:241-247.

National Academy of Sciences (US) FaNB, Institute of Medicine, National Academies. 2011. Dietary reference intakes (dris). Available: https://www.nationalacademies.org/hmd/~/media/Files/Activity%20Files/Nutrition/DRI-Tables/5Summary%20TableTables%2014.pdf?la=en [accessed Jan 24 2017].

Navas-Acien A, Francesconi KA, Silbergeld EK, Guallar E. 2011. Seafood intake and urine concentrations of total arsenic, dimethylarsinate and arsenobetaine in the US population. Environmental Research 111:110-118.

Nermell B, Lindberg AL, Rahman M, Berglund M, Persson LA, El Arifeen S, et al. 2008. Urinary arsenic concentration adjustment factors and malnutrition. Environmental Research 106:212-218.

Nijhout HF, Reed MC, Ulrich CM. 2008. Mathematical models of folate-mediated one-carbon metabolism. Vitamins and Hormones 79:45-82.

Pierce BL, Argos M, Chen Y, Melkonian S, Parvez F, Islam T, et al. 2011. Arsenic exposure, dietary patterns, and skin lesion risk in Bangladesh: A prospective study. Am J Epidemiol 173:345-354.

Pu YS, Yang SM, Huang YK, Chung CJ, Huang SK, Chiu AW, et al. 2007. Urinary arsenic profile affects the risk of urothelial carcinoma even at low arsenic exposure. Toxicology and Applied Pharmacology 218:99-106.

Rodrigues EG, Bellinger DC, Valeri L, Hasan MO, Quamruzzaman Q, Golam M, et al. 2016. Neurodevelopmental outcomes among 2- to 3-year-old children in Bangladesh with elevated blood lead and exposure to arsenic and manganese in drinking water. Environmental Health : A Global Access Science Source 15:44.

Sattar A, Xie S, Hafeez MA, Wang X, Hussain HI, Iqbal Z, et al. 2016. Metabolism and toxicity of arsenicals in mammals. Environmental Toxicology and Pharmacology 48:214-224.

Schlebusch CM, Gattepaille LM, Engstrom K, Vahter M, Jakobsson M, Broberg K. 2015. Human adaptation to arsenic-rich environments. Mol Biol Evol 32:1544-1555.

Schoof RA, Yost LJ, Eickhoff J, Crecelius EA, Cragin DW, Meacher DM, et al. 1999. A market basket survey of inorganic arsenic in food. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 37:839-846.

Steinmaus C, Carrigan K, Kalman D, Atallah R, Yuan Y, Smith AH. 2005a. Dietary intake and arsenic methylation in a u.S. Population. Environmental Health Perspectives 113:1153-1159.

Steinmaus C, Yuan Y, Kalman D, Atallah R, Smith AH. 2005b. Intraindividual variability in arsenic methylation in a u.S. Population. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 14:919-924.

Steinmaus C, Bates MN, Yuan Y, Kalman D, Atallah R, Rey OA, et al. 2006. Arsenic methylation and bladder cancer risk in case-control studies in Argentina and the United States. Journal of Occupational and Environmental Medicine 48:478-488.

Styblo M, Del Razo LM, Vega L, Germolec DR, LeCluyse EL, Hamilton GA, et al. 2000. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Arch Toxicol 74:289-299.

Tsuji JS, Perez V, Garry MR, Alexander DD. 2014. Association of low-level arsenic exposure in drinking water with cardiovascular disease: A systematic review and risk assessment. Toxicology 323:78-94.

Vahter M. 2000. Genetic polymorphism in the biotransformation of inorganic arsenic and its role in toxicity. Toxicol Lett 112-113:209-217.

Vahter M. 2002. Mechanisms of arsenic biotransformation. Toxicology 181-182:211-217.

Vahter M, Bjorkman L, Goessler W. 2007. Concentrations of biomarkers in spot urine samples need adjustment for variation in dilution--comment on: "Distribution of urinary selenium and arsenic among pregnant women exposed to arsenic in drinking water" [environ res. 2006;100(1):115-122]. Environmental Research 104:312-313; discussion 314.

Valenzuela OL, Borja-Aburto VH, Garcia-Vargas GG, Cruz-Gonzalez MB, Garcia-Montalvo EA, Calderon-Aranda ES, et al. 2005. Urinary trivalent methylated arsenic species in a population chronically exposed to inorganic arsenic. Environmental Health Perspectives 113:250-254.

Wang W, Xie Z, Lin Y, Zhang D. 2014. Association of inorganic arsenic exposure with type 2 diabetes mellitus: A meta-analysis. Journal of Epidemiology and Community Health 68:176-184.

Wu H, Grandjean P, Hu FB, Sun Q. 2015. Consumption of white rice and brown rice and urinary inorganic arsenic concentration. Epidemiology 26:e65-67.

Xue J, Zartarian V, Wang SW, Liu SV, Georgopoulos P. 2010. Probabilistic modeling of dietary arsenic exposure and dose and evaluation with 2003-2004 NHANES data. Environmental Health Perspectives 118:345-350.

Yager JW, Greene T, Schoof RA. 2015. Arsenic relative bioavailability from diet and airborne exposures: Implications for risk assessment. Sci Total Environ 536:368-381.

Zablotska LB, Chen Y, Graziano JH, Parvez F, van Geen A, Howe GR, et al. 2008. Protective effects of b vitamins and antioxidants on the risk of arsenic-related skin lesions in Bangladesh. Environmental Health Perspectives 116:1056-1062.

Table 1. Urinary As biomarkers, limits of detection (LOD), percent below LOD.

		LOD[a]	< LOD[a]
Total As (As)		0.74 µg/l	
Arsenobetaine (AsB)	AsB	0.4 µg/l	[<40%]
Arsenic acid (As(V))	As(V)	1.0 µg/l	92.4%
Arsenous acid (As(III))	As(III)	1.2 µg/l	95.6%
Inorganic As (inAs)	As(III) + As(V)		
Monomethylarsonic acid (MMA)	MMA(III + V)	0.9 µg/l	65.0%
Dimethylarsinic acid (DMA)	DMA(III + V)	1.7 µg/l	[<40%]
Sum of As species (sumAs)	As(III) + As(V) + MMA + DMA		
inAs%	inAs/sumAs		
MMA%	Primary Methylation Index (PMI)		
DMA%	DMA/sumAs		
DMA/MMA	Secondary Methylation Index (SMI)		

[a]Urinary As LODs are from Caldwell et al., 2009. Values below the LOD were imputed as follows: LOD/√2.

Table 2. Dietary arsenic, nutrient intake and nutrient status in the U.S. population, NHANES 2003-2004, stratified by race/ethnic group.

	Total Population	Non-Hispanic White	Mexican-American	Other Hispanic	Non-Hispanic Black	Other Race	Overall p-value
N observations (weighted %)	2420 (100)	1023 (71.0)	586 (8.7)	65 (3.7)	658 (11.7)	88 (5.0)	
DIETARY INTAKE							
Seafood (yes)							
weighted proportion	0.14	0.13	0.09	0.18	0.17	0.28	0.035
Rice-eater (yes)							
weighted proportion	0.13	0.09	0.12	0.28	0.21	0.46	0.001
Dietary total As (μg/day)							
GM	41.08	37.32	41.02	70.15	48.53	79.62	<0.001
SE	1.03	1.04	1.05	1.13	1.08	1.14	
Dietary inAs (μg/day)							
GM	6.20	5.82	6.49	9.91	6.19	10.32	<0.001
SE	1.02	1.02	1.03	1.15	1.04	1.08	
Total folate (μg/day)							
GM	333.35	336.36	342.22	302.41	310.03	363.91	0.019
SE	1.02	1.02	1.02	1.06	1.03	1.04	
Vitamin B12 (μg/day)							
GM	3.53	3.63	3.55	2.71	3.15	3.56	0.002
SE	1.03	1.03	1.07	1.16	1.04	1.19	
Vitamin B6 (mg/day)							
GM	1.51	1.50	1.62	1.39	1.50	1.66	0.106
SE	1.02	1.03	1.03	1.05	1.03	1.05	
Total protein (gm/day)							
GM	70.97	70.58	74.28	68.06	71.32	73.91	0.140
SE	1.01	1.01	1.02	1.05	1.03	1.08	
NUTRIENT STATUS							
RBC folate (ng/ml)							
GM	256.33	269.65	239.11	231.31	210.67	232.27	<0.001
SE	1.02	1.02	1.02	1.15	1.02	1.06	

Serum folate (ng/ml)							
GM	12.00	12.62	10.78	10.15	9.92	10.95	<0.001
SE	1.02	1.03	1.02	1.05	1.03	1.07	
Serum vitamin B12 (pg/ml)							
GM	472.82	451.02	523.72	478.63	547.27	516.89	0.005
SE	1.02	1.03	1.02	1.07	1.03	1.07	
Plasma vitamin B6 (nmol/l)							
GM	43.04	45.39	38.62	37.51	34.24	42.44	0.131
SE	1.05	1.07	1.11	1.14	1.08	1.18	
Plasma tHcys (µmol/l)							
GM	7.94	8.27	6.52	7.55	7.37	7.26	<0.001
SE	1.02	1.02	1.02	1.05	1.02	1.08	
Plasma methylmalonic acid (µmol/l)							
GM	0.13	0.14	0.11	0.12	0.11	0.13	<0.001
SE	1.02	1.03	1.02	1.06	1.03	1.07	

GM, geometric mean; SE, standard error (population weighted); p-value based on F-statistic

Table 3. NHANES 2003-2004 population subset, stratified by race/ethnic group: Urinary biomarkers, geometric means and standard errors.

Urinary biomarkers	Total Population Sample	Non-Hispanic White	Mexican-American	Other Hispanic	Non-Hispanic Black	Other Race	Overall p-value
Creatinine (mg/dL)							
GM	100.91	95.87	107.18	96.12	140.41	95.45	<0.001
SE	1.03	1.04	1.03	1.09	1.02	1.15	
Total As (µg/l)							
GM	8.30	7.14	9.12	13.21	11.79	17.10	0.001
SE	1.07	1.07	1.06	1.12	1.09	1.22	
Sum of As species (µg/l)							
GM	6.52	5.97	7.62	9.48	7.10	9.64	0.001
SE	1.04	1.04	1.04	1.09	1.04	1.08	
inAs%							
Mean	27.05	28.71	23.59	20.69	24.72	19.60	0.001
SE	0.79	0.82	0.82	1.77	0.72	1.34	
MMA%							
Mean	14.11	14.72	13.79	11.41	12.52	11.82	0.006
SE	0.23	0.21	0.54	0.75	0.70	0.81	
DMA%							
Mean	58.84	56.57	62.62	67.91	62.75	68.58	<0.001
SE	0.87	0.82	0.61	2.21	1.30	1.46	
DMA:MMA							
GM	4.42	4.03	4.85	6.76	5.44	6.34	0.001
SE	1.03	1.03	1.04	1.13	1.08	1.10	

GM, geometric mean; SE, standard error (population weighted); p-value based on F-statistic

Table 4. Crude predictors of urinary biomarkers of As exposure (regression coefficients).

	inAs%	MMA%	DMA%	DMA/MMA[a]
Female				
β	3.20	-0.32	-2.88	-0.02
P-value	<0.001	0.467	0.013	0.451
Age				
β	0.04	-0.01	-0.03	-0.00
P-value	0.014	0.130	0.094	0.868
BMI				
β	-0.02	-0.09	1.03	0.003
P-value	0.738	0.007	0.114	0.030
Current smoker				
β	0.532	1.39	-1.92	-0.06
P-value	0.421	<0.003	0.015	0.001
Dietary total As[a,b]				
β	-10.10	-3.59	13.7	0.25
P-value	<0.001	<0.001	<0.001	<0.001
Dietary inAs[a,b]				
β	-10.47	-2.41	12.88	0.19
P-value	0.005	0.002	<0.001	<0.001
Seafood (yes/no)				
β	-7.62	-3.49	11.12	0.22
P-value	<0.001	<0.001	<0.001	<0.001
Rice (yes/no)				
β	-5.44	-1.99	7.43	0.13
P-value	<0.001	0.004	0.001	0.007
Urine creatinine[a]				
β	-22.6	-3.24	25.87	0.35
P-value	0.001	<0.001	<0.001	<0.001

β, regression coefficient; [a]log(10)-transformed; [b]dietary As and nutrient intake adjusted for energy intake

Table 5. Crude relation between dietary and blood nutrients and urinary As biomarkers in children (<18 years)[c] and adults (≥18 years)[d].

	inAs%		MMA%		DMA%		DMA/MMA[a]	
	Children	Adults	Children	Adults	Children	Adults	Children	Adults
Dietary total folate[a,b]								
β	-2.01	-4.94	-1.94	-0.08	3.95	5.02	0.09	0.05
P-value	0.462	0.018	0.107	0.891	0.195	0.014	0.085	0.095
Dietary vitamin B6[a,b]								
β	-3.19	-3.84	-0.83	0.35	4.02	3.49	0.05	0.01
P-value	0.189	0.039	0.493	0.650	0.074	0.100	0.260	0.703
Dietary vitamin B12[a,b]								
β	-1.74	-1.24	0.26	-0.14	1.49	1.38	0.01	0.01
P-value	0.287	0.396	0.785	0.885	0.450	0.540	0.806	0.852
Dietary total protein[a,b]								
β	-4.48	-4.65	-0.94	-0.49	5.42	5.14	0.07	0.05
P-value	0.136	0.051	0.417	0.602	0.057	0.066	0.135	0.374
RBC folate[a]								
β	-5.36	1.54	-4.01	-0.19	9.37	-1.35	0.18	-0.02
P-value	0.334	0.695	0.063	0.905	0.166	0.798	0.112	0.874
Serum folate[a]								
β	-4.86	2.65	-4.21	1.48	9.07	-4.13	0.20	-0.08
P-value	0.194	0.310	0.105	0.063	0.123	0.192	0.093	0.089
Plasma vitamin B6[a]								
β	-2.89	1.06	0.79	0.70	2.09	-1.76	0.00	-0.04
P-value	0.179	0.143	0.393	0.186	0.427	0.098	0.946	0.064
Serum vitamin B12[a]								
β	0.48	-0.69	-1.44	0.64	0.95	0.05	0.05	-0.02
P-value	0.839	0.802	0.320	0.332	0.733	0.987	0.429	0.611
Plasma tHcys[a]								
β	-2.88	3.78	6.65	0.47	-3.77	-4.25	-0.21	-0.07
P-value	0.407	0.244	0.020	0.704	0.342	0.301	0.026	0.285

Plasma methylmalonic acid[a]								
β	-2.66	5.10	3.22	-0.37	-0.56	-4.73	-0.09	-0.05
P-value	0.492	0.061	0.216	0.711	0.900	0.146	0.377	0.376

β, regression coefficient; [a]log(10)-transformed; [b]dietary nutrient intake adjusted for energy intake; [c]number of children varied between 662 and 785 for different analytes; [d]number of adults varied between 1527 and 1631 for different analytes

Table 6. Nested multivariable regression of urinary As biomarkers. Blocks: 1) dietary As, 2) confounders, 3) urine creatinine, 4) nutrient intake, 5) blood nutrients, 6) interaction effects.

Blocks	Covariates	InAs% β (95% CI)	MMA% β (95% CI)	DMA% β (95% CI)	DMA/MMA[a] β (95% CI)
1	Dietary total As (μg/day)[a,b]	-7.15 (-10.16,-4.14)	-2.02 (-3.03,-1.00)	9.20 (5.55,12.85)	0.16 (0.10,0.22)
	Seafood (yes)	-2.94 (-5.14,-0.75)	-2.21 (-3.10,-1.32)	5.11 (2.65,7.57)	0.12 (0.06,0.17)
	Rice (yes)	-1.77 (-3.41,-0.13)	-0.41 (-1.26,0.44)	2.14 (-0.12,4.40)	0.03 (-0.03,0.09)
2	Sex (female)	-0.90 (-2.24,0.44)	6.88 (3.07,10.69)	-5.83 (-14.00,2.34)	-0.27 (-0.43,-0.10)
	Ethnicity/race				
	White, non-Hispanic (ref)	---	---	---	---
	Mexican-American	-3.96 (-6.42,-1.51)	-1.02 (-2.10,0.06)	4.99 (2.88,7.09)	0.07 (0.03,0.11)
	Other Hispanic	-5.87 (-9.17,-2.57)	-2.43 (-4.15,-0.70)	8.30 (3.79,12.82)	0.17 (0.06,0.28)
	Black, non-Hispanic	0.12 (-1.63,1.86)	-1.14 (-2.66,0.37)	1.01 (-1.32,3.34)	0.05 (-0.02,0.11)
	Other races, multi-racial	-5.23 (-7.58,-2.88)	-1.55 (-3.40,0.31)	6.78 (3.32,10.25)	0.10 (0.00,0.20)
	Current smoker (yes)	-0.07 (-1.19,1.06)	1.13 (0.18,2.08)	-1.06 (-2.69,0.56)	-0.05 (-0.09,-0.005)
	Age (years)	-0.07 (-0.10,-0.05)	-0.03 (-0.06,-0.01)	0.11 (0.06,0.15)	0.002 (0.001,0.003)
	Body mass index (BMI)	0.60 (0.16,1.04)	-0.39 (-0.12,0.05)	-0.74 (-1.39,-0.08)	-0.001 (-0.004,0.003)
3	Urine creatinine (mg/dL)[a]	-19.50 (-24.56,-14.43)	-1.81 (-3.58,-0.04)	19.07 (11.29,26.85)	0.31 (0.23,0.40)
4	Dietary Total protein (gm)[a,b]	2.77 (-1.42,6.96)	-0.25 (-3.33,2.83)	-2.54 (-8.51,3.43)	-0.03 (-0.18,0.12)
	Dietary total folate (μg)[a,b]	-0.96 (-4.50,2.58)	-0.10 (-1.95,1.76)	1.03 (-2.89,4.95)	0.02 (-0.06,0.10)
	Dietary vitamin B12 (μg)[a,b]	1.89 (0.78,3.00)	0.13 (-1.34,1.61)	-0.02 (-0.04,-0.002)	-0.03 (-0.08,0.03)
	Dietary vitamin B6 (mg)[a,b]	-3.69 (-6.42,-0.96)	0.67 (-0.84,2.19)	3.01 (-0.14,6.16)	0.00 (-0.06,0.07)
5	RBC folate (ng/mL RBC)[a]	-4.48 (-8.63,-0.33)	-1.24 (-4.13,1.64)	5.68 (-0.82,12.19)	0.08 (-0.11,0.26)
	Serum folate (ng/ml)[a]	-0.97 (-3.55,1.62)	1.39 (-0.43,3.21)	-0.41 (-4.07,3.25)	-0.03 (-0.13,0.06)
	Serum vitamin B12 (pg/ml)[a]	0.03 (-2.31,2.37)	-0.19 (-1.98,1.61)	0.18 (-2.57,2.92)	0.00 (-0.07,0.07)
	Plasma vitamin B6 (nmol/l)[a]	-0.36 (-1.26,0.54)	0.28 (-0.91,1.47)	0.08 (-1.54,1.70)	-0.01 (-0.06,0.04)
	Plasma tHcys (μmol/l)[a]	3.32 (-0.48,7.13)	3.33 (1.22,5.44)	-6.60 (-10.61,-2.59)	-0.16 (-0.25,-0.08)
	Plasma methylmalonic acid (μmol/l)[a]	0.86 (-1.55,3.27)	-1.61 (-4.59,1.37)	0.71 (-3.78,5.20)	0.04 (-0.07,0.16)
6	Sex (female) X urine creatinine[a]	---	-3.76 (-5.35,-2.17)	3.69 (0.14,7.24)	0.15 (0.08,0.22)
	BMI X urine creatinine[a]	-0.21 (-0.41,-0.01)	---	0.30 (0.02,0.58)	---

MODEL FIT:	ΔR^2	adjR^2	ΔR^2	adjR^2	ΔR^2	adjR^2	ΔR^2	adjR^2
Block 1: Dietary As intake	0.109***	0.010	0.066***	0.004	0.127***	0.016	0.109***	0.011
Block 2: Confounders	0.037*	0.019	0.036**	0.009	0.037**	0.026	0.036**	0.020
Block 3: Urine creatinine	0.401***	0.298	0.041***	0.019	0.334***	0.247	0.163***	0.094
Block 4: Dietary nutrient intake	0.007*	0.306	0.001	0.019	0.004*	0.251	0.002	0.094
Block 5: Blood nutrient status	0.008	0.314	0.008*	0.021	0.008*	0.258	0.006**	0.098
Block 6: Interaction effects	0.001*	0.315	0.010***	0.023	0.002*	0.261	0.006**	0.102

β (95% CI), regression coefficient (95% confidence interval); ΔR^2, change in R^2; adjR^2, adjusted R^2; *p<0.05; **p<0.01; ***p<0.001

[a]log(10)-transformed; [b]dietary As and nutrient intake adjusted for energy intake

Figure 1. One-carbon (1C) metabolic pathways and metabolism of inorganic arsenic (inAs).

InAs is metabolized in the liver via two metabolic pathways that comprise 1C metabolism: the folate cycle and the methionine cycle. These complex cycles involve folate, amino acids and B vitamins as well as various enzymes (not discussed here). Dietary folate is reduced to tetrahydrofolate (THF) and converted to 5-methyl-THF (mTHF) through a series of steps that utilize vitamin B6. Completing the folate cycle, mTHF is de-methylated and the carbon is donated to the methionine cycle, methylating homocysteine (hCys) for synthesis of methionine using vitamin B12 as a cofactor. The amino acid methionine is used to generate S-adenosylmethionine (SAM), which may undergo demethylation to form S-adenosylhomocysteine (SAH) and be converted back into hCys. Connected to the methionine cycle, the trans-sulfuration pathway involves conversion of hCys to cysteine, which can be used for synthesis of glutathione (GSH) (Locasale 2013). Metabolism of inAs utilizes SAM as a methyl donor for conversion of As^{III} to MMA^{V} and MMA^{III} to DMA^{V}, and requires GSH for reduction of the pentavalent forms (As^{V}, MMA^{V}, DMA^{V}) to their respective trivalent forms (As^{III}, MMA^{III}, DMA^{III}). (Additional abbreviations: DHF, dihydrofolate; me-THF, methylenetetrahydrofolate; F-THF, 10-formyltetrahydrofolate.)