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

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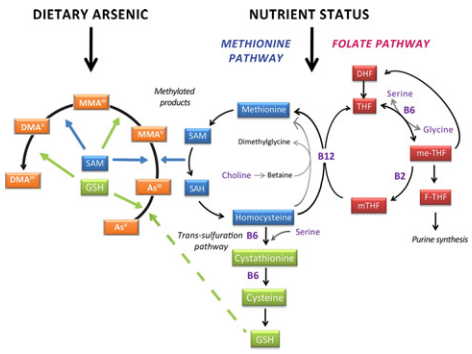
HIGHLIGHTS

- Majority of toxic arsenic exposure in the U.S. is from food.
- One-carbon nutrients are independently associated with urinary As metabolism.
- High metabolic levels of folate and dietary Vit B6 may mitigate inAs toxicity.
- High plasma tHcys and dietary Vit B12 may increase susceptibility to inAs exposure.
- NHANES results corroborate those seen in highly exposed, malnourished populations.

GRAPHICAL ABSTRACT



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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%

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(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.

1. 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) (Fig. 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 methylation to dimethylarsinic acid (DMA(V)), which may undergo further reduction to DMA(III). Other nutrients associated with the 1C pathway include protein, as a source of amino acids (methionine, cysteine, serine, glycine), and additional B vitamins, including B6 (Kile and Ronnenberg, 2008; Locasale, 2013; Nijhout et al., 2008; Vahter, 2002).

While total urinary As includes all As species, organic and inorganic, the sum of inAs and methylated As species excreted in urine (sumAs = As(III) + As(V) + MMA + DMA) is a frequently used biomarker of inAs exposure (Kalman et al., 1990). The proportion of each As species excreted in urine varies across individuals and reflects differences in exposure and As methylation capacity (Kile and Ronnenberg, 2008). The role of methylation in inAs detoxification is somewhat uncertain given that some of the trivalent intermediate methylation products show even greater toxicity than inAs (Agusa et al., 2011; Styblo et al., 2000; Vahter et al., 2007; Valenzuela et al., 2005). Methylation, however, increases the rate of whole body clearance of inAs (Drobna et al., 2010). Approximately 60–80% of inAs ingested is excreted in the form of DMA(III + V), 10–30% as inAs (As(III + V)), and 2–30% as MMA(III + V) (Loffredo et al., 2003; Schlebusch et al., 2015; Vahter, 2000). SumAs is used as the denominator in assessing differences in excretion patterns. Higher proportions of inAs and/or MMA are associated with increased As retention in tissues, lower excretion rates and increased susceptibility to toxicity (Pu et al., 2007; Schlebusch et al., 2015; Steinmaus et al., 2006; Vahter, 2000; Valenzuela et al., 2005). A higher proportion of DMA is associated with greater methylation efficiency and reduced toxicity. Variability in these ratios among populations has been attributed to heterogeneity in the As(III) methyl transferase (AS3MT) gene, diet, age, gender, body mass, nutritional status, and other factors (Chung et al., 2009; Engstrom et al., 2011; Gomez-Rubio et al., 2010; Hennig et al., 2012; Howe et al., 2014; Steinmaus et al., 2005b).

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

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

2. Methods

2.1. 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