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Low level arsenic exposure, B-vitamins, and achievement among Uruguayan school children

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Abstract

Objectives: Millions of children globally, including the U.S., are exposed to low levels of arsenic from water and food. Arsenic is a known neurotoxicant at high levels but its effects at lower exposure levels are understudied. Arsenic methylation capacity, influenced by B-vitamin intake and status, potentially influences arsenic toxicity. In a cross-sectional study of 5–8 year-old children from Montevideo, we assessed the relationship between urinary arsenic (U-As) and academic achievement, and tested for effect modification by B-vitamin intake, status, and arsenic methylation capacity.

Methods: Broad math and reading scores were calculated based on six subtests (calculation, math facts fluency, applied problems, sentence reading fluency, letter word identification, passage comprehension) from the Woodcock-Muñoz Achievement Battery. B-vitamin intake was assessed from two non-consecutive 24-hour dietary recalls, serum folate and vitamin B-12 levels were measured in a subset of participants. Arsenic methylation capacity was measured as the proportion of urinary monomethylarsonic acid (%MMA). Multiple imputation using chained equations was

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conducted to account for missing covariate and exposure data. Ordinal regressions assessed associations between U-As and achievement score tertiles in the complete case and imputed samples. A “B-vitamin index” was calculated using principal component analysis. Interactions by urinary %MMA and the B-vitamin index were assessed.

Results: Median specific gravity adjusted U-As was 11.7 µg/l (range: 2.6, 50.1). We found no association between U-As and broad math and reading scores, nor effect modification by %MMA or B-vitamins.

Conclusion: At low-levels of exposure, U-As does not appear to affect children’s academic achievement.

Keywords

Low level arsenic; B-vitamins; reading; math

Introduction

Arsenic exposure is a global public health concern (George et al., 2014). Millions of people worldwide, including the U.S., are exposed to low levels of arsenic from water and food (Focazio et al., 2000; Naujokas et al., 2013). Food is a particularly important source of arsenic exposure when water arsenic levels are low, as it is in much of the U.S. (Davis et al., 2012; Rey deCastro et al., 2014; US Food and Drug Administration, 2014). We know that children exposed to high levels of arsenic can experience cognitive deficits (Rosado et al., 2007; von Ehrenstein et al., 2007; Wasserman et al., 2007; Wasserman et al., 2004; Wasserman et al., 2011). On the other hand, the effects of low-level arsenic on children’s neurobehavioral outcomes are poorly understood (Wasserman et al., 2014). We also have little evidence to inform how low-level arsenic exposure affects learning and school achievement.

Previous work on lead highlights the importance of studying effects of toxicants on school achievement (Amato et al., 2012; Miranda et al., 2007; Zahran et al., 2009; Zhang et al., 2013). As an outcome, academic achievement has direct use in public health decision making. Greater academic success facilitates student transition into independent adult lives. The relationship between arsenic exposure and school achievement has only been studied in children with fairly high level of exposure. One study in Bangladesh (water arsenic mean, range: 119.5, 0.1–1263.2 µg/L) showed no effect of arsenic exposure on children’s performance on a national scholastic test (Khan et al., 2012). Urinary arsenic (mean ± SD, 58.1 ± 33.2 µg/L) was also not associated with performance on a math achievement test in Mexican first-graders (Rosado et al., 2007). For low-level arsenic exposure (urinary arsenic, median, range: 11.9, 1.4–93.9 µg/L), we observed no association with general intellectual abilities in first-graders (~7 year olds) in the Salud Ambiental Montevideo study in Uruguay (Desai et al., 2018). While academic achievement and intelligence are strongly correlated (Bartels et al., 2002; Jensen, 1998; Neisser et al., 1996; Sternberg et al., 2001), achievement depends on the child’s ability to utilize several cognitive domains, regulate behavior, and deploy social skills in peer and teacher interactions (Rimm-Kaufman and Chiu, 2007;

Sánchez-Pérez et al., 2018). These abilities are not reflected in intelligence scores, hence the need to further evaluate the effect of low-level arsenic on academic achievement.

The role of arsenic methylation capacity, B-vitamin intake and status as potential modifiers of arsenic neurotoxicity remains largely unexplored (Desai et al., 2018; Hamadani et al., 2011; Hsieh et al., 2014). Once in the body, arsenic undergoes two methylation cycles that convert inorganic arsenic to monomethylarsonous acid (MMA), followed by dimethylarsinic acid (DMA) (Challenger, 1945). The proportion of MMA excreted in the urine reflects the body's capacity to convert MMA to DMA. Higher %MMA and lower %DMA indicate lower methylation efficiency, which is a susceptibility factor for several arsenic-related health outcomes (Del Razo et al., 1997; Pu et al., 2007; Steinmaus et al., 2006; Vahter, 2001; Wu et al., 2006). Evidence from both observational studies and randomized clinical trials indicates that nutritional methyl donors, such as folate, influence the sequential methylation of inorganic arsenic to MMA and DMA, due to their role in synthesizing the universal methyl donor S-adenosylmethionine (Bozack et al., 2018; Gamble et al., 2006; Howe et al., 2014). Both intake and status of B-vitamins are associated with efficient arsenic methylation (Gamble et al., 2006; Gamble et al., 2005; Gamble et al., 2007; Hall et al., 2009a; Hall et al., 2009b; Howe et al., 2017; Steinmaus et al., 2005). Nevertheless, the role of B-vitamins in modifying arsenic-induced health outcomes is not well understood, particularly in populations with mandatory fortification.

The aim of the current study was to investigate the association between low-level arsenic exposure measured as the sum of urinary inorganic arsenic, MMA, and DMA, and academic achievement, thus expanding evidence beyond general intellectual functioning. We also tested arsenic methylation capacity (urinary %MMA) and dietary B-vitamin intake as potential effect modifiers. In a sub-sample of children, we explored whether serum folate and serum vitamin B-12 concentrations moderated the association between arsenic exposure and academic achievement.

The study site consisted of private elementary schools in Montevideo, Uruguay. Previous studies have shown that children in Montevideo are exposed to several toxic metals including lead, arsenic, cadmium, and manganese (Kordas et al., 2010; Mañay et al., 2008; Queirolo et al., 2010). Studies of young children have shown that exposure to metals co-occurs with anemia (Bradman et al., 2001; Wright et al., 1999). In Uruguay, commercially produced wheat flour is fortified with elemental iron and folate, but not other B-vitamins, since 2006. Assessing the role of low-level arsenic exposure on child development in such populations can provide insights into the potential role of diet in mitigating the adverse effects of arsenic.

Materials and Methods

Study Setting and Participant Recruitment

This study is part of research conducted between July 2009 and August 2013 in private elementary schools in neighborhoods of Montevideo considered to be at risk of pediatric metal exposure. The median arsenic concentration in drinking water in these areas was 0.45 µg/L (range: 0.10, 18.92), but there is evidence that this population is also exposed to arsenic

from foods such as rice (Kordas et al., 2010; Kordas et al., 2016). In this study, 357 children ~5–8 years and their mothers were enrolled. The details on recruitment can be found in a previous publication (Desai et al., 2018).

Measurements

Urinary Arsenic—Total urinary arsenic concentration was assessed as the sum of inorganic arsenic, MMA, and DMA in spot urine samples. Children collected their first void urine samples in cups rinsed with 10% nitric acid and deionized water. The samples were transported on ice to the Center for Research, Catholic University of Uruguay, Montevideo, within the day of collection and stored at -20°C in 10 mL plastic tubes previously rinsed with 10% nitric acid and deionized water, and further transported to the Karolinska Institutet, Stockholm, Sweden, for analysis. Arsenic methylation capacity was measured as urinary %MMA. Urinary arsenic concentration was measured using HPLC-HG-ICP-MS (HG, hydride generation, selects inorganic arsenic and its methylated metabolites into the ICP-MS, Inductively Coupled Plasma Mass Spectrometry), as described previously (Desai et al., 2018; Roy et al., 2015). The limit of detection was 0.1 $\mu\text{g/L}$ for inorganic arsenic (III) and MMA, 0.2 $\mu\text{g/L}$ for DMA, and 0.3–0.5 $\mu\text{g/L}$ for inorganic arsenic (V). The intra- and inter-assay coefficients of variation were ~4%. Seven urine samples (2.1%) were below limit of detection for inorganic arsenic (III) and 26 (7.9%) were below the limit of detection for inorganic arsenic (V). We used the measured values in statistical analyses. Urinary arsenic concentrations were adjusted for specific gravity to account for hydration status. This measure is referred to as U-As throughout this study.

B-vitamin Intake—Two 24-hour dietary recalls were conducted with the mother/caregiver, as described previously (Desai et al., 2018). The first recall was conducted on the same day as the blood and urine collection in school, and the second was conducted over the phone, at least two weeks later. The amounts of thiamine, riboflavin, niacin, vitamin B-6, folate, and vitamin B-12 in each food were calculated using the Uruguayan nutrient database or the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference, Release 28 (Version Current: September 2015) for foods not listed in the Uruguayan database (2018; INDA), 2010; Instituto de Nutrición de Centro América y Panamá (INCAP), 2012; Kordas et al., 2018). The foods listed as “unenriched” in the USDA database were used in these calculations, because foods in Uruguay are not fortified with B-vitamins other than folate. Beginning in 2006, all commercially produced wheat flour in Uruguay has been fortified with 30 mg of elemental iron and 2.4 mg of folic acid per kilogram. This fortification practice was taken into account while calculating the total folate intake. Estimated daily intake of the vitamins was the average of the two dietary recalls. Each B-vitamin intake was further adjusted for total energy intake and expressed per 1000 kcal/day.

Blood Collection—Fasting blood was collected by a phlebotomy nurse between 8 and 11 am when the child and a parent/guardian visited the school. Approximately 3 mL of venous blood was collected using a 25-gauge safety butterfly blood collection set (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) in heparin coated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for lead analysis. Additionally, 3 mL venous blood was

drawn into a serum tube with clot activator and separator gel (Becton Dickinson, Franklin Lakes, NJ). Serum samples were aliquoted for C-reactive protein, folate, vitamin B-12, and other assays. The whole blood, serum and urine samples were stored on ice in a cooler for the remainder of the clinic visit, and then transported to the respective laboratories until analysis.

Serum folate and serum vitamin B-12 assays—Serum folate and vitamin B-12 concentrations were measured at the Clinical Biochemistry and Molecular Diagnostics Research Laboratory at the University at Buffalo using the Food and Drug Administration 510(k) cleared diagnostic reagent kits, calibrators and quality control (QC) materials from Diazyme Laboratories (Poway, CA). Assays kits were a homogeneous, competitive enzyme method for the quantification of folate and vitamin B-12 antigens in human serum. QC consisted of a Diazyme two level control sets specific for each assay and were analyzed with each assay batch. Folate controls were at 2 and 4 ng/mL with coefficients of variation (CVs) of 15% and 9% respectively across all study batches. Vitamin B-12 controls were at 580 and 1100 pg/mL with CVs of 8% and 2% respectively across all study batches. The linear range (lower limit of quantification to upper limit of quantification) for serum folate and vitamin B-12 assays were 2.0 to 20.0 ng/mL and 96.7 to 2000 pg/mL respectively. The vitamin B-12 assay demonstrates a 0.98 correlation with the predicate Siemens vitamin B-12 Assay and folate demonstrates a 0.98 correlation with the predicate Roche Elecsys Folate III assay. The assays were performed on the ABX Pentra 400 automated chemistry analyzer (Horiba Instruments, Irvine, CA) using instrument application parameters provided by Diazyme specifically for the ABX Pentra 400.

Sample preparation and treatment was done according to manufacturer instructions (Laboratories, 2019a, b) where 95 µL of serum was required for each B12 assay and 50 µL for each folate assay in addition to 100 µL dead volume in the ABX Pentra autoanalyzer sample container. If the available sample volume exceeded 300 µL, both folate and vitamin B-12 assays were run in batches with B-12 assay being run first. If the sample volume was less than 300 µL, only the folate assay was completed as it was much more likely that we would have sufficient volume to complete this assay. Of the 307 available samples for folate assay, 32 had insufficient volume, and 5 were above the upper limit of quantification (20.0 ng/mL). Values above the upper limit of quantification were replaced with the highest observed values. This resulted in 275 observations with serum folate levels. Of the 306 available samples for vitamin B-12 assay, 54 had insufficient volume, and 23 were below the limit of quantification. Values below the limit of quantification were entered as the quantification limit divided by the square root of two. This resulted in 252 observations with serum vitamin B-12 levels.

Academic Achievement—Children participated in two separate neurobehavioral assessments. First, they were administered the Woodcock-Muñoz Cognitive Battery (Riverside Publishing, Rolling Meadows, IL), as described previously (Desai et al., 2018). We have previously published findings on the relationship between U-As and endpoints from the cognitive battery (Desai et al., 2018). During the second evaluation, the Woodcock-Muñoz Achievement Battery was administered to assess children's achievement in math and

reading. The batteries are co-normed: the cognitive and achievement measures were standardized on the same group of people, but, although related, are intrinsically different. The co-normed batteries enable the assessment of domain specific and general academic skills in relation to cognitive skills. Variation of scores between the batteries is possible, and may help in identifying discrepancies in performance. The Woodcock-Muñoz Achievement Battery and its English version, the Woodcock-Johnson Achievement Battery are widely used to assess academic achievement among children (Hughes et al., 2005; Liew et al., 2010; Liew et al., 2008). Studies have shown good reliability and validity for these batteries (Diamantopoulou et al., 2012; McGrew and Woodcock, 2006).

Six subtests from the Achievement Battery were administered: calculation, math facts fluency, applied problems, sentence reading fluency, letter word identification, and passage comprehension. Each subtest generated an age-and sex-scaled W score, which is based on the Rasch logit scale, an equal interval scale (McGrew and Woodcock, 2006). The W scores are based on a norming sample, which in this case consisted of children from Costa Rica, Mexico, Spain, Peru, and Puerto Rico (Rosselli et al., 2001). For each age group from the norming sample, a median W score is derived, that indicates the level of difficulty at which 50% of the sample responded correctly. This median W score is the reference value against which the performance of each participant is measured (Jaffe, 2009).

W scores from the calculation, math facts fluency, and applied problems subtests are averaged to yield the broad math domain score, and W scores from the sentence reading fluency, letter word identification, and passage comprehension subtest scores are averaged to yield the broad reading domain score. The two broad scores were the endpoints for this study.

Covariates—Trained nurses or nutritionists measured children's height in triplicate to the nearest 0.1 cm using a portable stadiometer (Seca 214, Shorr Productions, Colombia, MD), and weight in triplicate to the nearest 0.1 kg using a digital scale (Seca 872, Shorr Productions, Colombia, MD). Hemoglobin was measured using a portable hemoglobinometer (HemoCue Inc, Lake Forest, CA), and lead concentrations were measured by Atomic Absorption Spectrometry (AAS, VARIAN SpectrAA-55B) using flame or graphite furnace ionization techniques, in fasting venous blood samples. Details of these methods are in a previous publication (Desai et al., 2018). Parents completed questionnaires pertaining to the socio-demographic characteristics of the family, including questions about home ownership, possession of various items in the household, and crowding at home. A household possessions score was calculated based on a factor analysis that retained the ownership of five items – computer, car, refrigerator, laundry, and a landline telephone. The details of all these measures have been described previously (Desai et al., 2018). Home Observation for Measurement of the Environment (HOME) inventory score (Bradley et al., 2003), a measure of the availability of developmental stimulation in the child's home, was also measured, as detailed previously (Barg et al., 2018).

Statistical analyses

Descriptive analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA); medians (range) of U-As and broad math and reading scores were calculated in relation to participant characteristics. Descriptive analyses were conducted among the complete case sample of 239 participants, defined as having complete data on the exposure, outcomes, and covariates of interest. Multiple imputations using chained equations with fifty iterations were carried out in Stata 12.0 (StataCorp, College Station, TX, USA) for those participants who had data on both outcomes, resulting in an imputed sample size of 310. Multiple imputations conducted using chained equations require the specification of a separate conditional distribution for every imputed variable. Regression models are used to predict the missing values of variables, conditional on other variables. This is done through several iterations, starting with the variable with the least missing values, to the one with the most missing values (Azur et al., 2011).

Broad math and reading scores were split into tertiles because of their non-normal distributions. Ordinal regression using generalized estimating equations was used to assess the association between the continuous U-As and tertiles of the outcome in the complete-case (n=239) and imputed (n=310) samples, after the proportional odds assumption was met. Models were adjusted for season (to account for differences in the school year when the achievement tests were administered), sex, maternal education (years), household possessions score, HOME inventory score, blood lead, hair manganese, and hemoglobin concentrations. Models were further adjusted for school clusters. Non-linear relationship between U-As and the outcomes was assessed with a squared term for U-As. A principal component analysis (PCA) was used to create an index of the B-vitamins. PCA led to the separation of two indices – one with high loadings on all B-vitamins except folate (index 1), and the other with a high loading on folate only (index 2), possibly because of folate fortification of flour in Uruguay. As a next step, a factor score was calculated based on index 1, referred hereafter as the “B-vitamin index”. Urinary %MMA, the B-vitamin index, and folate intake were used as effect modifiers by creating an interaction term of each of these, stratified at the median, with U-As (continuous). Folate intake was assessed as a separate effect modifier because the B-vitamin index did not include folate. As an exploratory analysis, serum folate (n=222 in the complete case sample) and vitamin B-12 levels (n=221 in the complete case sample), measured in a subset of participants, were also used as effect modifiers in the same way. Models with interaction terms were adjusted for the same covariates as the main models. Interactions were assessed at a significance level of 0.05.

Results

The median (range) U-As concentration was 11.7 µg/L (2.6, 50.1). Energy adjusted median dietary intakes of thiamine, riboflavin, niacin, vitamin B-6, folate, and vitamin B-12 were 0.85 mg/1000 kcal, 0.99 mg/1000 kcal, 8.6 mg/1000 kcal, 0.68 mg/1000 kcal, 219.76 µg/1000 kcal, and 1.67 µg/1000 kcal respectively. The median concentration of serum folate was 11.40 ng/ml (correlation with dietary folate intake: 0.07), and that of serum vitamin B-12 was 489.45 pg/ml (correlation with dietary vitamin B-12 intake: 0.12). Urinary %MMA had a median (range) of 9.8% (2.6%, 24.8%).

The median (range) of U-As concentrations and broad math and reading scores according to participant characteristics are shown in Table 1. Supplemental Table 1 presents a description of the Woodcock-Muñoz Achievement Battery subtests and the means (SD) and medians (range) of the W scores. Supplemental Table 2 presents participant characteristics in the complete case sample according to tertiles of broad reading scores (participant characteristics by tertiles of broad math scores are very similar and are not presented).

Table 2 shows the likelihood of transitioning from one math and reading score tertile to the next in relation to U-As, based on ordinal regression. U-As was not associated with the likelihood of moving across the tertiles of math or reading achievement scores. For broad math, there was some indication of an association, however, in the direction that was not hypothesized (OR=1.02; 95% CI: 1.00, 1.04; $p=0.05$); every higher unit of U-As was associated with 2% higher odds of being in the upper tertile of math scores. Findings were similar in the complete-case and imputed samples, with overlapping confidence intervals. There was no evidence of a non-linear relationship between U-As and achievement (result not shown).

The results of interaction tests are presented in Table 3. Because serum folate and vitamin B-12 concentrations could only be assessed among a subset of the complete case sample, the sample size for the interaction between U-As and serum folate was 222, and that for serum vitamin B-12 was 221. An interaction was observed between U-As and serum vitamin B-12 concentrations with broad math score in the complete case, OR = 1.12 (95% CI: 1.02, 1.24), as well as the imputed sample, OR = 1.05 (95% CI: 1.02, 1.09).

Discussion

We found little evidence that low level arsenic exposure affected children's math or reading scores on the Woodcock-Muñoz Achievement Battery. In addition, there was no effect modification by urinary %MMA, the B-vitamin index, or dietary folate intake. Our exploratory analysis showed evidence of effect modification of the U-As-math achievement association by serum vitamin B-12; increased U-As and serum vitamin B-12 levels were associated with improved math performance. In a previous study, we found no effect of low-level arsenic exposure on general intellectual ability in these same children (Desai et al., 2018). In contrast, a recent study found an inverse association between low-level arsenic exposure and motor function in preschool children from Spain (Signes-Pastor et al., 2019), whereas several studies have shown inverse associations with intelligence and visual-spatial ability in geographical areas characterized by elevated arsenic exposure through drinking water (Nahar et al., 2014; Rosado et al., 2007; Wasserman et al., 2007; Wasserman et al., 2004). In Bangladesh, where arsenic exposure had been linked with intellectual ability deficits (Hamadani et al., 2011; Wasserman et al., 2007), arsenic in water was not related to achievement (Khan et al., 2012).

To our knowledge, the study from Bangladesh (Khan et al., 2012) represents the only other report on arsenic exposure and academic achievement in children. Our findings are consistent but few similarities exist between the two studies. First, arsenic exposure in Bangladesh was higher (water arsenic mean, range: Bangladesh, 119.5, 0.1–1263.2 $\mu\text{g/L}$ vs.

Uruguay, 0.63, 0.1–18.9 µg/L). Furthermore, academic achievement in Bangladesh was measured as annual scores on the national tests of English, Bangla, and math, whereas we assessed achievement using a standardized battery based on competencies children typically achieve by a certain age. Additionally, children from Bangladesh were older than children in our study (age 8–11 years in Bangladesh vs. 5–8 years in Uruguay). The scarcity of evidence, and the differences between the two studies underscore the need for further research on this topic. For example, behavior, social interactions, self-efficacy, parental involvement in schooling, and other factors within household and school predict school achievement; it would be important to understand how they contribute to achievement in the context of arsenic exposure.

The role of methylation capacity in arsenic neurotoxicity among children is not clear. Poor methylation capacity among Taiwanese children was a risk factor for developmental delays (Hsieh et al., 2014; Hsueh et al., 2016). The only two studies on arsenic and children's neurodevelopment that included stratification by %MMA did not show evidence of effect modification (Desai et al., 2018; Hamadani et al., 2011). One of these studies was conducted in Montevideo by our group (Desai et al., 2018). It is of note that no standard definition of "high" or "low" %MMA exists, perhaps because various factors affect methylation of arsenic, including the consumption of methylated arsenic species directly from the diet. We did not observe any effect modification by urinary %MMA in the current analyses, which is perhaps unsurprising given our previous findings on cognitive performance (Desai et al., 2018).

Because B-vitamins participate in arsenic methylation, we hypothesized that B-vitamin intake/status would modify the arsenic-achievement relationship. However, our findings did not support this hypothesis, possibly because of the low variability of B-vitamin intake observed in this population. The Recommended Daily Allowance (RDA) for 4–8 year olds is 0.6 mg/day for thiamine, riboflavin and vitamin B-6, 0.8 mg/day of niacin equivalents, 200 µg/day of dietary folate equivalents, and 1.2 µg/day of vitamin B-12 (Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference et al., 1998). Commercially produced wheat flour in Uruguay has been fortified with folate since 2006. In our sample, 68% and 48% of participants reported consuming various types of bread and cookies, respectively. Additionally, 99% of participants had intakes above the RDA for thiamine, riboflavin, niacin, vitamin B-6, and vitamin B-12; 90% exceeded the RDA for folate. Thus, participants in our study appeared to have largely adequate levels of B-vitamin intake, another potential reason behind the observed results. However, determining sufficiency/deficiency of vitamin intake on the basis of 24-hour recalls may not be accurate, thus caution is advised when interpreting these findings. We observed interaction effects between serum vitamin B-12 and U-As on broad math scores, indicating that higher U-As as well as serum vitamin B-12 were associated with better math performance. Overall, because U-As and serum vitamin B-12 were associated with lower likelihood of moving to into higher math score categories, the positive interaction term represents a fairly small effect. It is also important to note that we were unable to further explore this interaction in stratified analysis because the proportional odds assumption was not met in the serum B-vitamin strata, and the sample sizes were further reduced.

Arsenobetaine is an organic arsenical commonly found in fish, and is excreted from the body without undergoing any chemical changes (Navas-Acien et al., 2011). On the other hand, arsenolipids and arsenosugars, also found in seafood, are metabolized to DMA (Raml et al., 2005; Schmeisser et al., 2006). Rice is another important source of inorganic arsenic as well as DMA (Schoof et al., 1999; Signes-Pastor et al., 2016; Williams et al., 2005). Higher urinary DMA could result from efficient methylation of arsenic, or from consumption of seafood and to some extent rice. As a result, urinary %MMA is regarded as a more reliable marker of arsenic methylation capacity than urinary %DMA (Vahter, 2001). In our study, only 20 participants reported any consumption of seafood and about half of the participants reported any rice intake. Seafood intake may lead to increased urinary DMA, thereby overestimating total urinary arsenic. We re-analyzed our data by excluding the 20 participants with any seafood intake; the results did not change (Supplemental Table 3). Because seafood intake may also be associated with neurodevelopmental outcomes due to omega fatty acids (Oken and Bellinger, 2008), we then adjusted our models for seafood intake; the results did not change (Supplemental Table 4). We did not adjust the main models for rice intake because arsenic exposure was measured as the sum of urinary inorganic arsenic, MMA, and DMA; adjusting for rice would mean adjusting for the exposure itself. The borderline statistically significant positive association between urinary arsenic exposure and broad math score that we observed could be due to chance. Further, while it is likely that study was underpowered, even after imputation, it does provide effect size estimates in the context of low-level arsenic exposure.

Our study has certain limitations. First, we had a participation rate of 53%, which could have resulted in selection bias if participation was associated with both the exposure and outcome. Arsenic exposure is not routinely tested in Uruguay, so it is very unlikely that participation decisions would be made on that basis; therefore, the likelihood of selection bias is low. Second, of the 357 children who participated in our study, urinary arsenic measures were obtained from 327 children and academic achievement was assessed among 310 children. Although exclusion of children with missing information resulted in a complete case sample of 239 participants, we conducted multiple imputations using chained equations to impute covariates among those with complete data on the outcomes of interest, thereby increasing our sample to 310 participants. Thus, of these 310 participants, 77% (n=239) had complete data, and 23% (n=71) had data imputed for one or more variables. The use of a robust imputation technique, and the fact that we observed similar results in the imputed and the complete case sample increase confidence in our findings. Third, dietary intake of B-vitamins was derived from two different databases, including the USDA food composition database. Although we matched as closely as possible the foods consumed by the participants to those listed in the USDA database, measurement error is possible. We did not specifically query B-vitamin supplement use, but did ask whether participants took iron supplements because they are more commonly used for children. Only 6% participants reported any intake of iron supplements, leading us to believe that overall supplement use in this population would be low. Nonetheless, misclassification of B-vitamin intake due to supplement use is possible.

The strengths of this study include the use of the standardized Woodcock-Muñoz Achievement Battery to assess academic achievement, which makes our findings

comparable to others using this instrument (on the other hand, the use of country-specific assessments, while reflective of educational norms, makes comparison across studies challenging). Children are on their academic achievement trajectories by the end of their third grade and follow those until the end of schooling (Alexander et al., 1988). Our study captures an important part of this trajectory. In addition to the two 24-hour diet recalls, we had serum folate and vitamin B-12 levels, which is another strength. We adjusted our models for measures of lead and manganese exposure to account for their potential effects on the outcome, and HOME inventory scores to account for developmental inputs the child receives at home.

Conclusion

We found little evidence that low-level exposure to arsenic affects academic achievement among children attending first grade of school. We also found no impact of B-vitamin status, intake or methylation capacity on the relationship between urinary arsenic and achievement.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

DMA	dimethylarsinic acid
HOME	Home Observation for Measurement of the Environment
MMA	monomethylarsonous acid
PCA	Principal Component Analysis
U-As	specific gravity adjusted urinary arsenic
USDA	United States Department of Agriculture

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Table 1:

Specific gravity adjusted urinary arsenic (U-As) levels and test scores from the Woodcock Muñoz Achievement Battery by sociodemographic, dietary, anthropometric, and biochemical characteristics among ~7-year old children from Montevideo, Uruguay (n=239)

Covariates	N	U-As, µg/l ^{1,2}	Woodcock Muñoz Broad math ²	Achievement Battery, W score Broad reading ²
Age in months				
57 – 80	117	11.2 (2.6, 50.1)	457 (412, 486)	448 (378, 526)
81 – 105	122	12.3 (4.8, 46.1)	461 (407, 487) *	466 (382, 510) *
sex				
Boys	136	11.9 (2.6, 50.1)	458 (409, 487)	451 (382, 504)
Girls	103	11.5 (3.4, 42.0)	460 (407, 486)	462 (378, 526)
Maternal education				
Primary	41	12.8 (3.5, 39.7)	453 (411, 479)	438 (378, 496)
Secondary	148	11.3 (2.7, 50.1)	459 (407, 487)	458 (382, 506)
Tertiary	50	11.7 (2.6, 36.4)	461 (416, 486)	463 (389, 526)
>2 persons/bedroom				
Yes	53	11.9 (4.3, 50.1)	454 (409, 487)	444 (382, 505)
No	183	11.5 (2.6, 46.1)	460 (407, 486)	459 (378, 526)
Possessions score				
Below median	133	11.5 (2.7, 50.1)	459 (407, 486)	453 (382, 526)
Above median	106	12.0 (2.6, 46.1)	458 (411, 487)	458 (378, 502)
Drinking water source				
Tank/tap unfiltered	71	12.3 (2.6, 39.3)	457 (409, 478)	454 (382, 505)
Tap filtered	42	10.8 (4.1, 46.1)	461 (407, 477)	454 (384, 510)
Bottled/other	120	12.0 (3.5, 44.3)	459 (409, 487)	458 (378, 526)
Thiamine intake				
<0.85 mg/1000 kcal	117	11.2 (2.6, 42.0)	459 (409, 487)	461 (378, 510)
0.85 mg/1000 kcal	118	12.2 (3.4, 50.1)	458 (407, 486)	450 (382, 526)
Riboflavin intake				
<0.99 mg/1000 kcal	117	11.5 (2.6, 42.0)	459 (407, 487)	458 (378, 506)
0.99 mg/1000 kcal	118	12.0 (3.4, 50.1)	458 (409, 486)	451 (382, 526)
Niacin intake				
<8.6 mg/1000 kcal	117	11.0 (2.6, 44.3)	459 (409, 487)	458 (378, 526)
8.6 mg/1000 kcal	118	12.5 (3.4, 50.1)	458 (407, 486)	451 (382, 504)
Vitamin B-6 intake				
<0.68 mg/1000 kcal	117	11.2 (2.7, 50.1)	459 (407, 487)	462 (378, 526)
0.68 mg/1000 kcal	118	12.3 (2.6, 46.1)	458 (409, 486)	452 (382, 510)
Folate intake				
<219.76 µg/1000 kcal	117	12.3 (3.5, 46.1)	457 (409, 486)	456 (378, 510)
219.76 µg/1000 kcal	118	11.2 (2.6, 50.1)	459 (407, 487)	455 (382, 526)
Vitamin B-12 intake				

Covariates	N	U-As, µg/l ^{1,2}	Woodcock Muñoz Broad math ²	Achievement Battery, W score Broad reading ²
<1.67 µg/1000 kcal	117	11.9 (2.6, 50.1)	459 (407, 487)	457 (378, 506)
1.67 µg/1000 kcal	118	11.6 (3.4, 46.1)	458 (409, 486)	455 (382, 526)
Height for age z score				
<0.34	120	11.2 (2.6, 50.1)	460 (411, 487)	456(378, 506)
0.34	119	12.0 (3.4, 46.1)	457 (407, 486)	457(382, 526)
Weight for age z score				
<0.64	121	11.2 (3.5, 50.1)	459 (409, 487)	450 (378, 505)
0.64	118	12.0 (2.6, 46.1)	459 (407, 486)	462 (382, 526)
Blood lead				
<3.8 µg/dL	118	11.8 (2.6, 42.0)	459 (407, 486)	462 (378, 526)
3.8 µg/dL	121	11.7 (4.1, 50.1)	458 (409, 487)	450 (384, 505)
Hemoglobin				
<13.1 g/dL	114	11.6 (3.7, 50.1)	459 (407, 486)	456 (384, 526)
13.1 g/dL	125	11.9 (2.6, 46.1)	459 (409, 487)	458 (378, 510)
Hair manganese				
<0.85 ppb	119	11.3 (3.4, 50.1)	460 (411, 487)	457 (386, 526)
0.85 ppb	120	12.2 (2.6, 46.1)	457 (407, 486)	456 (378, 510)
Serum folate ^{**}				
<11.40 ng/ml	93	11.0 (2.7, 50.1)	468 (409, 486)	455 (382, 526)
11.40 ng/ml	99	12.4 (2.6, 46.1)	457 (407, 487)	457 (386, 506)
Serum vitamin B-12 ^{**}				
<489.45 pg/ml	82	13.5 (2.6, 46.1)	459 (407, 487)	460 (384, 526)
489.45 pg/ml	82	10.7 (2.7, 50.1)	457 (409, 479)	460 (382, 510)

¹ Adjusted for urinary specific gravity

² Value given as median (range)

* Statistically significant difference at an alpha value of 0.05, based on Mann-Whitney U test

** Reduced sample sizes because the laboratory analyses were conducted in a subset of the sample.

Table 2:

Association between arsenic exposure^a and math and reading scores based on ordinal regression analysis

Achievement score	Model 1 ¹ OR (95% CI)	Model 2 ² OR (95% CI)	Model 3 ³ OR (95% CI)
Broad math score			
Complete case sample (n=239)	1.01 (0.99, 1.04)	1.01 (0.98, 1.04)	1.01 (1.00, 1.02)
Imputed sample (n=310)	1.02 (1.00, 1.05)	1.02 (1.00, 1.05)	1.02 (1.00, 1.04)
Broad reading score			
Complete case sample (n=239)	1.01 (0.98, 1.04)	1.00 (0.98, 1.03)	1.00 (0.98, 1.02)
Imputed sample (n=310)	1.01 (0.98, 1.03)	1.00 (0.98, 1.03)	1.00 (0.98, 1.02)

^aModeled as specific gravity adjusted urinary arsenic

¹Crude

²Adjusted for season, sex, maternal education, household possessions, HOME score, hemoglobin, blood lead concentrations, hair manganese concentrations

³Further adjusting for school clusters; achievement scores divided into tertiles; the OR represents the likelihood of moving from lower to higher tertile of achievement endpoint.

Table 3:

Interaction between specific gravity adjusted urinary arsenic and urinary %MMA, serum folate, serum vitamin B-12 and intake of B-vitamins, with tertiles of broad reading and math scores as the outcomes

	OR (95% CI) – complete case sample		OR (95% CI) – imputed sample	
Achievement score	Broad math	Broad reading	Broad math	Broad reading
Methylation capacity				
<i>%MMA main effect^a</i>	1.71 (0.78, 3.71)	1.75 (0.58, 5.32)	1.40 (0.53, 3.67)	1.60 (0.79, 3.27)
<i>U-As main effect</i>	1.02 (1.00, 1.04)	1.02 (0.99, 1.05)	1.04 (1.02, 1.06)	1.01 (0.98, 1.05)
<i>Interaction</i>	0.98 (0.95, 1.01)	0.98 (0.95, 1.01)	0.99 (0.95, 1.04)	0.99 (0.94, 1.03)
B vitamin intake				
<i>B-vitamin index^b main effect</i>	0.98 (0.48, 2.01)	0.51 (0.25, 1.02)	1.17 (0.67, 2.03)	0.56 (0.26, 1.22)
<i>U-As main effect</i>	1.01 (0.99, 1.02)	1.00 (0.98, 1.02)	1.03 (1.00, 1.06)	0.99 (0.95, 1.02)
<i>Interaction</i>	1.00 (0.98, 1.03)	1.01 (0.98, 1.05)	1.00 (0.98, 1.03)	1.03 (0.98, 1.08)
<i>Folate intake^b main effect</i>	1.03 (0.35, 2.94)	1.27 (0.65, 2.48)	0.90 (0.34, 2.40)	1.15 (0.60, 2.22)
<i>U-As main effect</i>	1.00 (0.98, 1.02)	1.01 (0.98, 1.04)	1.02 (0.98, 1.06)	1.01 (0.97, 1.04)
<i>Interaction</i>	1.02 (0.98, 1.07)	1.00 (0.97, 1.03)	1.03 (0.99, 1.06)	1.01 (0.97, 1.04)
B vitamin status				
<i>Serum folate^c main effect</i>	0.56 (0.21, 1.52)	0.51 (0.30, 0.86)	0.58 (0.29, 1.18)	1.15 (0.60, 2.22)
<i>U-As main effect Interaction</i>	0.99 (0.98, 1.00)	0.98 (0.95, 1.00)	1.01 (0.98, 1.04)	1.00 (0.97, 1.04)
<i>interaction</i>	1.03 (0.99, 1.08)	1.05 (1.00, 1.10)	1.05 (1.02, 1.08)	1.00 (0.97, 1.04)
<i>Serum vitamin B-12^d main effect</i>	0.14 (0.02, 1.08)	0.62 (0.11, 3.59)	0.71 (0.27, 1.83)	1.46 (0.28, 7.56)
<i>U-As main effect</i>	0.95 (0.91, 1.00)	0.98 (0.94, 1.02)	1.01 (0.99, 1.04)	1.00 (0.95, 1.06)
<i>Interaction</i>	1.12 (1.02, 1.24)	1.04 (0.96, 1.13)	1.05 (1.02, 1.09)	1.01 (0.92, 1.11)

^a n=239 among the complete case sample, n=310 among the imputed sample

^b n=235 among the complete case sample, n=310 among the imputed sample

^c n=222 among the complete case sample, n=310 among the imputed sample

^d n=221 among the complete case sample, n=310 among the imputed sample

* indicates statistical significance at an alpha value of 0.05.

Models adjusted for season, sex, maternal education, household possessions, HOME score, hemoglobin, blood lead concentrations, hair manganese concentrations, and school clusters; achievement scores divided into tertiles; the OR represents the likelihood of moving from lower to higher tertile of achievement endpoint.