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A cross-sectional study of general cognitive abilities among Uruguayan school children with low-level arsenic exposure, potential effect modification by methylation capacity and dietary folate

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Abstract

Background—Few studies have evaluated the association between low-level arsenic (As) exposure and cognitive performance among children.

Objectives—In this cross-sectional study, we assessed the association between low-level As exposure and cognitive performance among 5–8 year-old children in Montevideo, and tested effect modification by As methylation capacity and children's dietary folate intake.

Methods—We measured total urinary As (UAs) concentrations and the proportion of monomethylarsonic acid (MMA) in the urine of 328 children. Seven subtests of the standardized Woodcock-Muñoz cognitive battery were used to assess cognitive performance, from which, the general intellectual abilities (GIA) score was derived. Total folate intake was estimated from two 24-hour dietary recalls. Linear regression analyses were performed. Effect modification was assessed by stratifying at the median %MMA value and tertiles of total folate intake calculated as micrograms (μg) of dietary folate equivalents (dfe).

Results—The median UAs was 11.9 μg/l (range = 1.4–93.9), mean folate intake was 337.4 (SD=123.3) μg dfe, and median %MMA was 9.42 (range=2.6–24.8). There was no association

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Conflicts of interests

The authors declare no conflicts of interests

between UAs and cognitive abilities, and no consistent effect modification by %MMA. UAs was associated inversely with concept formation, and positively with cognitive efficiency and numbers reversed subtest in the lowest folate intake tertile; UAs was also positively associated with sound integration in the second tertile and concept formation in the highest tertile of folate intake. There was no consistent pattern of effect modification by %MMA or folate intake. Conclusion: There was no association between low-level As exposure and general cognitive abilities.

Keywords

Low-level arsenic; folate; methylation capacity; cognition; children

Introduction

Arsenic (As) is a metalloid that occurs naturally in the earth's crust, and is released into air, water, and soil through natural as well as anthropogenic activities [1]. Human exposure to As and its compounds occurs primarily through ingestion of As-contaminated food and water [1]. About 200 million people worldwide are exposed to As levels in water exceeding the World Health Organization (WHO)-recommended limit of 10 μg/L [2]. Exposure to As is related to adverse health outcomes, including skin lesions, cardiovascular diseases, as well as cancer [3–9].

Among children, exposure to As is associated with deficits in intelligence quotient (IQ), cognitive development, and neurobehavioral function, although the evidence has been inconsistent [10–18]. For instance, a cross-sectional component of the Health Effects of Arsenic Longitudinal Study (HEALS) in Bangladesh showed that among 201 children aged 9.5 – 10.5 years with mean urinary As levels of 116.6 μg/L (SD=148.8), higher urinary As was inversely associated with performance and full-scale IQ, but not verbal IQ [17]. Another cross-sectional analysis, also of HEALS data, showed that exposure to As from water (mean water As levels=120.1 μg/L, SD=134.4, mean urinary As levels=110.7 μg/L, SD=132.8) was inversely associated with raw scores of performance IQ as well as processing speed among 301 6-year old children [16]. There was no association between water As concentrations and verbal IQ [16]. In contrast, urinary As with mean levels of 78.09 μg/L (SD=72.16) was inversely associated with verbal IQ in a third cross-sectional HEALS study, conducted among 299 8–11 year old children, even after adjusting for blood manganese levels [18].

Among 1700 young children, a longitudinal study estimated in utero exposure to As by measuring maternal, and later, the child's postnatal urinary As concentrations [15]. This study found that urinary As, measured as the sum of inorganic arsenic (iAs) and its methylated metabolites - monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA), was weakly inversely associated with both verbal IQ and full-scale IQ in 5-year old girls (effect size calculations showed that 100 μg/l U-As was associated with a decrement of 1–3 points in both verbal IQ and full scale IQ), whereas no such association was observed among boys [15]. The median UAs was 80 μg/L (10–90 percentiles: 25–400 μg/L) among pregnant women, and 51 μg/L (20–238 μg/L) among children [15]. A systematic review concluded that there is an inconsistent association, and a weak dose-response relationship between exposure to relatively low levels of As, most commonly found to be below 100

μg/L in the drinking water, and neurological outcomes in children [19]. These inconsistencies can be attributed to the differences in study designs, sample sizes, and tests used to measure cognitive performance. It is important to note that very few studies have examined the association between low-level As and cognitive performance. In one study, U.S. children aged 8 – 10 y who had well water As concentrations ≥5μg/L scored 5 points lower on full scale IQ score than children with water As <5 μg/L [20].

Inorganic arsenic in the human body is metabolized to form MMA, followed by DMA [19]. Rapid formation and excretion of DMA in the urine is an indicator of the metabolism, and hence, internal exposure [21]. High %MMA in urine indicates poor methylation capacity of individuals, and higher internal exposure. The major factors that hamper the efficiency of methylation of iAs to DMA are age, smoking, sex, nutritional status, and reduced liver function [22–25]. Assessing potential effect modification by %MMA values in urine is indicative of the role of methylation capacity in relation to cognitive outcomes, and hence the use of %MMA as a susceptibility factor.

Very few studies on As exposure and cognitive performance have incorporated detailed measures of children's nutritional status. Calderon et al. conducted a study in Mexico that focused on the association between exposure to lead, As, undernutrition and neuropsychological development in children [10]. Height for age, a measure of growth, was positively associated with full-scale as well as performance IQ ($p<0.01$) [10]. Previous studies have shown that folate intake plays an important role in the metabolism of As in the body, thereby influencing the levels of As metabolites excreted in urine [26, 27]. As stated above, the methylation cycles that occur once inorganic As enters the human body result in the sequential production of MMA and DMA [28]. In these reactions, S-adenosylmethionine is a methyl donor, and its regeneration from S-adenosylhomocysteine requires methyl groups, donated by dietary folate or other dietary sources [21, 27]. Thus, folate plays an important role in both the methylation reactions of As. Results from randomized trials among adults show that folate may reduce blood As levels [27, 29]. Several studies have also indicated that folate is independently associated with cognitive functioning in adults [30–32]. The role of folate in one-carbon metabolism, which is required in the methylation reactions in the central nervous system and associated with neurocognitive performance, is hypothesized to underlie the folate-cognition association [33, 34]. Although the interaction between folate status and lead has been studied in relation to cognition among children [35], few studies have assessed the role of folate in the association between children's As exposure and cognitive performance. Understanding the role of folate intake is essential as it could be used in mitigating the cognitive deficits due to As exposure.

We address these gaps by examining the cross-sectional association between low level As exposure, as measured by total urinary As (UAs), and cognitive performance among first-grade children in Montevideo, Uruguay. Additionally, we assessed potential effect modification of these associations by dietary folate intake and urinary %MMA (a purported indicator of methylation capacity).

Materials and Methods

Study Setting

We conducted this study in Montevideo, the capital of Uruguay. Montevideo has several industries, including an oil refinery and has an interweave of several heavily travelled motorways. The residents of Montevideo have been exposed to several metals [36–38], previous studies showed a median As concentration in drinking water of 0.45 μg/L (5%–95% range: 0.16–0.93). The specific sources of As exposure are not well studied, but foods such as rice are an important contributor [36, 39]. Groundwater is not a source of drinking water for most Montevideo residents, and the municipal water authority carefully monitors water contaminant levels. We carried out this study between July 2009 and August 2013 in private elementary schools in several neighborhoods of Montevideo considered at risk of pediatric metal exposure.

Participant Recruitment

We contacted schools in the focal neighborhoods, and upon receiving the school director's affirmative response, scheduled meetings with the children's parents. After explaining the study rationale and procedures, we obtained parental consent. All first-grade children who regularly attended the participating schools were eligible. The sole exclusion criterion was a previous diagnosis of lead poisoning (defined as blood lead levels >45 μg/dL). None of the children were excluded based on this criterion. Of the 673 eligible children from 11 private elementary schools that agreed to participate, 357 children aged 5–8 years and their mothers enrolled into the study. The research protocol was approved by the Institutional Review Boards at the Catholic University of Uruguay, Pennsylvania State University, and the State University of New York at Buffalo.

Measurements

Anthropometry—Trained pediatric 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).

Parental questionnaires—Caregivers filled out questionnaires about socio-demographic characteristics of the family, child's medical history, and home environment. Questions regarding the monthly income, daily expenditures on food and clothes, home ownership, crowding at home, as well as family possessions of household items were included to assess household socioeconomic status. A household possessions score was based on a rotated factor analysis that retained the ownership of five items – computer, car, refrigerator, laundry, and a landline telephone to calculate the final score. Due to low response rates to income and expenditure questions, the possessions score was used as a proxy for socioeconomic status in statistical models. To identify possible sources of environmental exposures, questions about the type of housing, frequency of dusting or cleaning at home, sources of drinking and cooking water were also included in the questionnaire.

Urine analysis—Children collected their first void urine samples in cups rinsed with 10% HNO3 and deionized water that had been previously provided to them. 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% HNO3 and deionized water, and further transported to the Karolinska Institutet, Stockholm, Sweden, for analysis. Exposure to As was measured based on the urinary concentration of iAs, MMA and DMA. HPLC-HG-ICP-MS (HG, hydride generation, selects inorganic arsenic and its methylated metabolites into the ICP-MS, Inductively Coupled Plasma Mass Spectrometry) were used to measure the concentration of As. The metabolites of iAs, i.e., arsenite [As(III)], arsenate [As(V)], MMA, and DMA were separated using the Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany), with an anion exchange column (Hamilton PRP X-100,10 mm, 250 X 4.6 mm) and 10 μL injection volume. The LC separation was online with HG and ICP-MS (Agilent 7500ce, Agilent Technologies, Tokyo, Japan and operated as described previously [40, 41]. Standard solutions of the four As species were prepared from sodium arsenite (Purum p.a., ≥99.0%; Fluka Chemika, Switzerland), sodium hydrogenarsenate hepahydrate (98 + %, A.C.S. reagent, Aldrich Chemical Company, WI, USA), sodium dimethylarsinate trihydate (Merck, Schuchardt, Germany), and disodium methylarsenate hexahydrate (>97.5%, Supelco, Bellefonte, PA, USA). The working standard solutions (one for each arsenic metabolite) were gravimetrically prepared fresh daily for 7-point calibration curves. The limit of detection (LOD) was 0.1 μg/L for inorganic As (III) and MMA, 0.2 μg/L for DMA, and 0.3–0.5 μg/L for inorganic As (V). The intra- and inter-assay coefficients of variation were ~4%. Seven urine samples (2.1%) were below LOD for inorganic As (III) and 26 (7.9%) were below LOD for inorganic As (V). We used the measured values in statistical analyses. Urinary specific gravity was measured using a portable specific gravity refractometer (PAL 10S, Atago Inc, USA) on the day of the sample collection, to account for variation in urinary sample dilution. These methods have been published previously [42].

Serum ferritin analysis—Fasting venous blood was collected by a phlebotomy nurse at the school using a 25-gauge safety butterfly blood collection set (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), into a serum tube with clot activator and separator gel (Becton Dickinson, Franklin Lakes, NJ). The tubes were left to stand for 45 minutes, centrifuged for 10 minutes at 3000 rpm, and aliquoted. Serum samples were shipped on dry ice to the Department of Nutritional Sciences, Pennsylvania State University to be stored at −20°C until analysis. Serum ferritin concentrations were determined in duplicate using one of two methods, according to manufacturer instructions: 1) an immunoradiometric assay (Coat-A-Count Ferritin IRMA; SIEMENS Diagnostic Products, USA) and 2) an enzyme immunoassay (Spectro Ferritin, RAMCO Laboratories, Texas, USA). Intra- and inter-assay coefficients (CV) were 4.2% & 9.5% respectively for the IRMA method and 1.7% and 7.6% respectively for the ELISA method. The use of different assays was addressed by deriving a correction factor, with the IRMA method serving as gold standard, and both values being log-transformed prior to the derivation step, and back-transformed for the main analysis.

Hemoglobin measurement—Hemoglobin was measured at the time of the blood draw in a drop of venous blood that was removed from the serum tube immediately after the draw.

A portable hemoglobinometer (HemoCue Inc, Lake Forest, CA) was used to measure hemoglobin, with quality control checks performed every day with standard HemoCue controls (low, medium, high) provided by the manufacturer.

Blood lead analysis—Approximately 3 ml of venous blood was also collected into heparin coated trace-metal free tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for lead analysis. Blood lead concentrations were measured at the Toxicology Laboratory "CEQUIMTOX" (Specialized Center for Chemical Toxicology), of the Faculty of Chemistry, University of the Republic of Uruguay by Atomic Absorption Spectrometry (AAS, VARIAN SpectrAA-55B) using flame or graphite furnace ionization techniques, depending on the volume of whole blood available. The detection limit was 1.8 μg/dL for the flame and 0.8 μg/dL for graphite furnace AAS techniques.

Dietary intake of folate—Two 24- hour dietary recalls were conducted by five trained nutritionists with the mother or a caregiver familiar with the child's diet. All the foods were assigned a unique code and entered, along with the amounts consumed, into a database that contained the nutrient composition of typical Uruguayan foods and preparations. Beginning in 2006, all commercially produced wheat flour has been fortified with 2.4 mg of folic acid and 30 mg of elemental iron per kilogram. Total dietary folate intake was calculated based on averaging the 24- hour dietary recalls and the fortification practices as micrograms (μg) of dietary folate equivalents (dfe), and adjusted for total energy intake using the residual method recommended by Willett [43].

Cognitive Performance—The Woodcock-Muñoz (Riverside Publishing, Rolling Meadows, IL) cognitive battery was used to assess cognitive performance. This was chosen because it is a standardized test that has Spanish norms available, suitable to the population of interest. The test included seven subtests: verbal comprehension, visual-auditory learning, spatial relations, sound integration, concept formation, visual matching, and numbers reversed. Each subtest generated a raw score, which was converted to percentile scoring. The age- and sex-scaled general intellectual abilities (GIA) score, verbal ability, thinking ability as well as cognitive efficiency scores were derived from a combination of the subtest scores. Supplementary Table 1 (Table S1) gives a detailed description of the Woodcock-Muñoz cognitive battery tests.

Statistical analyses

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The distribution of %MMA was slightly skewed, thus, participants were stratified at the median %MMA value of 9.42% to form "low" and "high" %MMA groups. Total folate intake (μg dfe) was categorized to form tertiles, which roughly approximates low, medium and high folate intakes. The exposure variable was the UAs concentration. Since it was not normally distributed, it was natural log-transformed. The outcome variables included the scores for the seven Woodcock-Muñoz sub-tests: verbal comprehension, visual-auditory learning, spatial relations, sound integration, concept formation, visual matching, numbers reversed, as well as the derived scores for verbal ability, thinking ability, cognitive efficiency, and the GIA. All these were normally distributed.

Separate linear regression analyses were performed for the exposure variable with each outcome. These models were first adjusted for school clusters at the design stage. As a next step, the models were adjusted for the child's sex, BMI-for-age z-score, serum ferritin, blood lead concentration, hemoglobin, season, test administrator, mother's education (primary, secondary, university or higher), crowding at home (having more than 2 persons per bedroom - yes/no), HOME inventory score, current parental smoking (yes/no), source of drinking water (tank/tap unfiltered, tap filtered, bottled with other source) and household possessions (above median/below median). These covariates were chosen a priori based on existing literature on cognitive performance of school-age children exposed to As [10, 12, 15, 44]. The HOME inventory score [45] is a measure of developmental stimulation available in the child's home environment. This was a combination of scores collected in two ways: during a home visit made by study staff, and a brief questionnaire version filled out by the parents during study meetings. The visit-based HOME inventory score was missing for 35 participants, who did have the questionnaire-based scores. In order to carry out imputation for the visit-based HOME score, we ran a simple linear regression analysis with the visit-based score as the dependent variable and the questionnaire-based score as the independent variable. Using the regression coefficient and the intercept obtained this way, we calculated a visit-based HOME score for the 35 participants with missing information (correlation 0.4).

We conducted further analyses by adjusting all models for rice consumption (derived from 24-hour recalls and coded as low or high consumption), to account for dietary sources of DMA, which could mask poor methylation capacity. We had previously found that higher rice consumption in this group of children was associated with higher %DMA [39]. We also conducted these analyses by further adjusting for urinary cadmium concentrations. We assessed interaction by sex by creating an interaction term between sex and the natural-log-transformed total UAs variable. Assessment of %MMA as a susceptibility factor was carried out by conducting these regression analyses separately in the "low" and "high" %MMA group. Similarly, effect modification by folate intake was assessed by conducting the linear regression analyses separately in the folate intake groups.

Results

The sociodemographic, anthropometric, and biochemical characteristics of the participants are shown in table 1. The median age of participants in this study was 81 months (range: 57–105), and approximately 55% of the children were boys. About 21% of the participants lived in houses classified as "crowded", and 50% of the households relied on a combination of bottled and other sources of water for drinking. The mean total folate intake from all sources was 337.4 (SD=123.3) μg dfe. Analysis of urinary As showed mean %DMA, %MMA and %iAs values to be 78.9% (SD=7.4), 9.7% (SD=3.6) and 11.5% (SD=5.8) respectively. The median UAs concentration was 11.9 μg/l (range = 1.4–93.9).

The distribution of the scores on the Woodcock-Muñoz cognitive battery tests by high versus low UAs levels is shown in Supplementary Table 2 (Table S2). The relationships between UAs and the cognitive measures are given in Table 2, showing no associations, either in the

crude or the fully adjusted models. There was no evidence of interaction by sex for any of the cognitive measures.

These results remained unchanged upon further adjustment for rice consumption and urinary cadmium concentrations (data not shown). Table 3 shows the results stratified by %MMA. Among the low %MMA group (defined as ≤9.42% and denoting efficient methylators), the scores for the numbers reversed test were higher by 5.76 units (95% CI: 2.04, 9.47; $p<0.05$) for every unit increase in the natural log-transformed urinary As, i.e. every 2.71-fold increase in urinary As. Similar positive associations were seen with visual-auditory learning (β=1.57, 95% CI: 1.15, 1.99) and cognitive efficiency (β=2.65, 95% CI: 0.01, 5.28). Among children with high %MMA (>9.42%; inefficient methylators), higher UAs was associated with higher visual matching scores (β=1.08, 95% CI: 0.03, 2.13). Concept formation showed an inverse association with UAs among the high %MMA group (β=−4.43, 95% CI: −8.09, −0.77).

The associations between folate intake and cognitive performance are presented in supplementary table 3 (Table S3). There was no association between low folate intake (≤ 297 μg dfe) and cognitive measures. Folate intake > 297 – ≤ 377 μg dfe was associated positively with the scores on verbal comprehension (β=0.07, 95% CI: 0.00, 0.13), visual-auditory learning (β=0.10, 95% CI: 0.04, 0.15), GIA scores (β=0.05, 95% CI: 0.00, 0.09), and verbal ability (β=0.07, 95% CI: 0.00, 0.13) tests. When folate intake was > 377 μg dfe, it was associated inversely with scores of visual-auditory learning (β=−0.04, 95% CI: −0.07, −0.02), and positively with concept formation (β=0.04, 95% CI: 0.01, 0.07), numbers reversed (β=0.07, 95% CI: 0.04, 0.10), and cognitive efficiency (β=0.04, 95% CI: 0.02, 0.06).

Table 4 shows the associations between UAs and cognitive measures, stratified by tertiles of total folate intake. Overall, there was little consistency in findings across the strata or among the tests, and of the 33 covariate-adjusted models performed, only 5 were statistically significant. UAs was associated inversely with concept formation (β= −6.27, 95% CI: −10.84, −1.70), and positively with the scores of numbers reversed subtest (β= 5.54, 95% CI: 1.95, 9.14) and cognitive efficiency (β= 2.72, 95% CI: 0.55, 4.89) when folate intake was low (≤ 296.8 μg dfe). There was a positive association between UAs and sound integration scores (β= 5.44, 95% CI: 2.38, 8.50) in the second tertile (folate intake > 296.8 – ≤ 377.3 μg dfe) and concept formation (β= 3.56, 95% CI: 1.56, 5.56) in the highest tertile (folate intake > 377.3 μg dfe).

Discussion

The main findings of this study were as follows: (i) There was no independent association between UAs and any of the measures of general cognitive ability; (ii) Although methylation capacity (expressed as high vs low %MMA) modified the association between UAs and cognitive performance, there were no clear or consistent patterns; (iii) Folate intake also modified the association between UAs and children’s cognition, but again, in an inconsistent manner. Our study did not show any associations between UAs and cognitive outcomes measured by the tests from Woodcock-Muñoz cognitive battery, perhaps because the

children in our study had much lower As exposure, mainly from dietary sources, compared to most other study samples. A study of school children in Maine, U.S. which found IQ differences between children with well water As concentrations ≥5μg/L compared to children with water As <5 μg/L, reported that about 30% of water samples exceeded 10 μg As/L, with highest observed value of 115.3 μg/L [20]. In our study, the highest water As value was 18.9 μg/L and the highest UAs (when adjusted for specific gravity) was 48.7 μg/L.

In the human body, As is metabolized when arsenite (As^{III}) is methylated to form monomethylarsonic acid (MMA^{V}), which is then reduced to form monomethylarsonous acid (MMA^{III}). A second methylation cycle converts MMA^{III} to dimethylarsinic acid (DMA^{V}) [28]. These methylation reactions are catalyzed by As methyltransferase (AS3MT), wherein S-adenosylmethionine (SAM) acts as a methyl donor, and the biosynthesis of SAM depends on availability of methyl groups, a common source of which is dietary folate and choline, among others [27, 46]. As a result, the individual variation in the methylation of As, which is regarded as a detoxification process, is influenced by folate status, choline status, and the status of other dietary sources of the methyl group [29]. Plasma folate, homocysteine, and red blood cell folate are usually used as biomarkers of the folate status of an individual, and are shown to be reliably reflective of the changes in folate intake [47–49]. Selenium also plays an important role in As metabolism, since it binds to and modifies AS3MT, thereby inhibiting the methylation process [25, 50, 51]. Measures of selenium intake were not collected as part of our study, which could be considered a limitation.

Two possible mechanisms have been hypothesized to explain the association between folate and cognition. Methylation reactions, wherein folate is the methyl group donor, convert homocysteine to methionine, which further produces SAM [52, 53]. SAM is required in many reactions important to cognitive functioning, such as production of myelin [53]. Folate deficiency thus results in the inhibition of processes important to neurologic status, such as methylation reactions involving proteins, DNA, membrane phospholipids, metabolism of dopamine, serotonin [33, 34]. Another hypothesis suggests that folate plays an important role in cognitive functioning because it is a key component of the cerebrovasculature [34]. Folate deficiency usually results in elevated levels of homocysteine, which affects the vascular tissue and causes vascular disease [34, 54]. Folate protects the central nervous system from vascular damage, thereby maintaining cognitive abilities [33, 34, 54, 55].

Folate intake has been shown to mitigate the inverse associations between lead exposure and cognitive performance among children [35]. Results from randomized trials have shown that folic acid supplementation enhances As methylation [29, 56]. The Recommended Dietary Allowance (RDA) for folate for 4–8 year old children ranges from 200 μg dfe to 400 μg dfe; the tolerable upper intake level of 400 μg dfe is based on the synthetic forms of folate, i.e., from fortified foods and dietary supplements [57]. Conversely, excess folate from food sources has not been reported to cause adverse health outcomes [57]. In our study sample, 11.5% of the participants had total folate intake <200 μg dfe. We found that fortified foods contributed substantially (61.4%) to total folate intake, and based on the consumption of fortified foods alone, 33% children appeared to have excessive intake. In comparison, >95% of 1–3-year-old U.S. children had folate intake more than the estimated average requirement from foods alone [58]. We stratified our analyses by creating tertiles of total folate intake, to

approximate intakes ranging from inadequate to sufficient. When we examined the association of folate with cognitive performance, we observed positive associations with tests measuring verbal abilities and cognitive efficiency. On the other hand, we found little consistent association between arsenic exposure and cognitive performance across the dietary folate strata.

Exposure to As is hypothesized to affect the brain cells by causing oxidative stress [59, 60]. Arsenic also acts as an endocrine disruptor, and interacts with estrogen and thyroid hormones, which are essential for neurodevelopment [61, 62]. Other mechanisms by which As affects cognition include inflammation [63], angiogenesis [64], as well as endothelial dysfunction [65]. These mechanisms have been shown with water As concentration as low as 50 μg/L in rats, and 0.01 μM sodium arsenite concentrations in human embryonic NT2 cells [60, 61]. These concentrations are still higher than the median water As level of 0.45 μg/L (range: 0.10 – 18.92) in our study. Studies on As exposure among humans have shown the presence of oxidative stress markers among exposed individuals [66, 67]. Specifically, a study on As exposure and oxidative stress has shown the presence of oxidative stress biomarkers, namely lipid peroxides and nonprotein sulfhydryl, among individuals exposed to high (mean water As=410 μg/L) as well as low levels (mean water As=20 μg/L) [68]. Although these studies suggest a possible mechanism of action of As, there is no evidence of the association between As exposure and cognition for concentrations as low as those in our study.

Considerable inter-individual variation has been observed in the metabolism of As [69]. The percentage of MMA excreted in the urine reflects the efficiency of the individual to convert MMA to DMA. Thus, a higher %MMA and a lower %DMA in urine is indicative of higher retention of MMA in the body, and a lower methylation efficiency [21]. High urinary %DMA concentrations can be seen as a result of the intake of foods such as rice and seafood, which are sources of DMA [39, 70]. As a result, urinary %MMA is a more reliable susceptibility factor for As-related health outcomes. Hence, the urinary %MMA is regarded as a susceptibility factor for As-related health outcomes [21]. Ours is one of the few studies to examine the association between As exposure and cognitive performance as a function of As methylation capacity. Studies of other health outcomes have shown urinary %MMA as a susceptibility factor. For instance, among Mexican adults, those with As-related skin lesions had higher %MMA compared to those without skin lesions [71]. Similar results were seen for urothelial carcinoma [72] and bladder cancer [73]. A study in Taiwan showed a 5.4- fold (95% CI: 2.0, 15.0) higher risk of atherosclerosis among those with higher urinary %MMA compared to those with low %MMA [74].

We found positive associations between UAs levels and visual-auditory learning, numbers reversed tests, and cognitive efficiency among those with low urinary %MMA. Among those with high urinary %MMA, there were no associations between these variables. In contrast, concept formation was inversely, while visual matching was positively associated with UAs among those with high urinary %MMA, and no association was observed among those with a low %MMA. These results are contradictory to the few studies using percent methylated species in relation to neurodevelopmental outcomes [15, 75]. For instance, a study in China showed that children with developmental delays had significantly higher urinary %MMA

levels compared to those without developmental delays [75]. However, this study focused on developmental delays, rather than normative variations in performance, thus precluding direct comparisons to our study. Few other studies of cognitive outcomes have shown stratification by urinary %MMA, and have found no differences in cognitive outcomes in the stratified groups [15]. As discussed above, several factors may influence individuals' methylation capacity, and the consumption of methylated As species from food may mask low methylation efficiency. When we adjusted our models for rice consumption (an important dietary source of DMA), our conclusions remained unchanged. Further investigation is needed in children exposed to low-level As, particularly, prospectively investigating the role of dietary sources of methyl groups such as choline and the B-vitamins, assessing more specific cognitive domains, using biomarkers of long-term As exposure and biomarkers of nutrient status instead of dietary intake data to reduce misclassification, and finally, understanding potential threshold effects and the long-term effects of exposure to low-level As.

The strengths of our study include the use of %MMA as a susceptibility factor, which informs us about the effects of methylation efficiency on cognitive outcomes in relation to As exposure. Since folate plays an important role in the metabolism of As in the body, the assessment of folate intake as an effect modifier in our study is also an important strength. We adjusted our models for rice intake, one of the major sources of dietary DMA, which is another important strength of our study. Furthermore, this is one of the first studies to focus on the association between low-level As exposure (water As <20 μg/L and urinary As <93.9 μg/L; <48.7 μg/L upon adjusting for specific gravity) and cognitive outcomes among children. We included measures of blood lead levels and the HOME inventory score, which have not been used in majority of similar studies to date. We also adjusted for other potentially influential variables including season of data collection, test administrator, BMI for age, iron status, and family socioeconomic status. The use of the standardized Woodcock-Muñoz cognitive battery is another strength. Cognitive functions and performance in humans develop well into adolescence and beyond [76]; our study captures an important period in that trajectory.

There are also some limitations to our study. First, of the 673 children who attended first-grade in the participating schools and were, therefore, eligible for the study, 357 (53%) participated. This level of participation could have resulted in selection bias if the participation was associated with the exposure and outcome, however, there is no way to measure this with certainty. With opt-in enrollment, we could not easily assess reasons for non-participation. Furthermore, lack of informed consent from non-participating families prevented us from collecting any information that would allow us to assess potential differences in exposure, outcome, or demographic characteristics. When we began the study in 2009, epidemiological or environmental health research in Montevideo schools was not common, and it is likely that parents chose not to participate because they were unfamiliar with the research process, rather than for other reasons.

With a sample of 353 children (324 children with data on the required covariates), stratification by folate intake and %MMA affected the statistical power to detect modest associations. The absence of a measure of folate status (as opposed to the intake variable we

used in our analysis) also limits the interpretation of our results; the collection of dietary intake data is prone to recall errors and may result in misclassification. Furthermore, dietary recall does not typically account for the bioavailability of nutrients due to the presence of inhibitors or promoters of absorption, or disease status of individuals. Finally, depending on the type of biomarker measured, biomarkers of status reflect more closely than dietary intake levels the amount of nutrient stored in the body or available for exchange or interaction with tissues. Higher intake of DMA through diets is associated with an apparent low %MMA, which could have been possible in our study as well. However, rice is one of the most important sources of dietary DMA, which we took into account. Other potential sources remain unaccounted for. While we believe that it is unlikely that cognitive outcomes would impact arsenic exposure (reverse causality), the cross-sectional nature of this study prevents us from establishing a temporal sequence and hence a causal association between As exposure and cognitive performance. Since we collected urine samples to measure As exposure only once, we lack serial exposure data.

Conclusion

We found no significant associations between low-level As exposure and cognitive outcomes in our study population, and inconsistent effect modification by %MMA and folate intake on select measures. Longitudinal studies with larger sample sizes are necessary to better understand the relationship between As exposure and cognition among children exposed to low-level As.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Sociodemographic, anthropometric, and biochemical characteristics of the Uruguayan schoolchildren in the study

Variables	N	% missing	All participants
Sociodemographic			
Age in months (median, IQR)	351	0.6	81 (8)
Child's gender	353	0.0	
Male (n, %)			195, 55.2%
Female (n, %)			158, 44.8%
Mother's education	334	5.4	
Primary (n, %)			65, 19.5%
Secondary (n, %)			206, 61.7%
Baccalaureate or higher (n, %)			63, 18.9%
More than 2 persons/bedroom	306	13.3	
Yes (n, %)			65, 21.2%
No (n, %)			241, 78.8%
Household possessions score	308	12.7	
Below median (n, %)			170, 55.2%
Above median (n, %)			138, 44.8%
Source of drinking water	303	14.2	
Tank/tap unfiltered (n, %)			94, 31.0%
Tap filtered (n, %)			57, 18.8%
Bottled with other source (n, %)			152, 50.2%
Folate intake, μg dfe *	321	9.07	
From fortified foods and supplements (mean, SD)			345.3 (158.3)
From dietary sources (mean, SD)			130.3 (50.1)
Total folate from all sources (mean, SD)			337.4 (123.3)
<200 dietary folate equivalents (n, %)			37, 11.5%
Anthropometric			
Height for age, z score (mean, SD)	324	8.2	0.47 (1.1)
Weight for age, z score (mean, SD)	324	8.2	0.78 (1.3)
BMI for age, z score (mean, SD)	324	8.2	0.69 (1.3)
Overweight (n, %)			130, 40.1%
Obese (n, %)			57, 17.6%
Biochemical			
Blood lead, μg/dL (median, IQR)	315	10.8	3.8 (2.1)
Hemoglobin, g/dL (median, IQR)	322	8.8	13.1 (1.3)
Urinary DMA, μg/l (median, IQR)	324	8.2	9.5 (8.5)
Urinary MMA, μg/l (median, IQR)	324	8.2	1.3 (1.0)
Urinary iAs, μg/l (median, IQR)	324	8.2	1.3 (0.8)
Total urinary As, μg/l (median, IQR)	324	8.2	11.9 (9.8)
%DMA (mean, SD)	324	8.2	78.9 (7.4)

Variables	N	% missing	All participants
%MMA (mean, SD)	324	8.2	9.7 (3.6)
% iAs (mean, SD)	324	8.2	11.5 (5.8)

* Not adjusted for energy intake

Table 2

Associations between As exposure* (μg/L) and child performance on the Woodcock-Muñoz cognitive battery.

	Model 1[1]: β (95% CI)	Model 2[2]: β (95% CI)	Interaction term[2, 3]
Verbal comprehension	0.004 (−1.14, 1.15)	0.04 (−1.64, 1.73)	−1.43 (−5.04, 2.19)
Visual-auditory learning	1.07 (−0.07, 2.21)	0.52 (−1.19, 2.23)	−0.60 (−3.72, 2.52)
Spatial relations	−0.15 (−0.79, 0.50)	0.20 (−1.18, 1.59)	−2.19 (−5.32, 0.95)
Sound integration	2.61 (−1.74, 6.96)	2.04 (−0.88, 4.96)	0.21 (−7.70, 8.11)
Concept formation	−0.38 (−2.56, 1.80)	−1.22 (−4.36, 1.92)	−0.98 (−4.71, 2.74)
Visual matching	0.32 (−0.90, 1.54)	−0.08 (−0.62, 0.46)	−0.45 (−5.03, 4.14)
Numbers reversed	−0.07 (−5.03, 4.90)	−0.04 (−5.74, 5.66)	5.35 (−3.62, 14.33)
GIA score	0.36 (−1.01, 1.73)	−0.05 (−1.91, 1.82)	0.27 (−2.90, 3.44)
Verbal ability	0.02 (−1.12, 1.16)	0.04 (−1.64, 1.73)	−1.43 (−5.04, 2.19)
Thinking ability	0.33 (−1.23, 1.89)	−0.19 (−1.68, 1.29)	−1.43 (−5.03, 2.17)
Cognitive efficiency	1.68 (−2.40, 5.76)	0.10 (−2.94, 3.14)	2.68 (−2.06, 7.41)

*Total urinary As adjusted for specific gravity and then natural log transformed.

[1]adjusted for school clusters at the design stage.

[2]adjusted further for child's sex, BMI for age z scores, serum ferritin, blood lead, hemoglobin, season, test administrator, mother's education, crowding at home, HOME inventory score, parental smoking, source of drinking water, and household possessions.

[3]interaction term consisting of sex*specific gravity adjusted and natural log transformed total urinary As

Table 3

Associations between As exposure* (μg/L) and child performance on the Woodcock-Muñoz cognitive battery – stratified by %MMA.

	Low %MMA β (95% CI)		High %MMA β (95% CI)	
	Model 1[1]	Model 2[2]	Model 1[1]	Model 2[2]
Verbal comprehension	−0.16 (−1.75, 1.43)	0.94 (−1.30, 3.18)	1.08 (−0.75, 2.92)	0.20 (−3.43, 3.82)
Visual-auditory learning	1.55 (0.58, 2.52)	1.57 (1.15, 1.99)	1.37 (−0.67, 3.41)	−0.67 (−2.37, 1.04)
Spatial relations	−0.37 (−1.48, 0.75)	0.34 (−0.17, 0.85)	0.53 (−2.06, 3.12)	0.271 (−3.18, 3.71)
Sound integration	0.79 (−1.18, 2.75)	2.75 (−0.92, 6.41)	6.31 (−1.23, 13.85)	−0.31 (−5.78, 5.16)
Concept formation	1.40 (−1.01, 3.82)	2.04 (−0.85, 4.93)	−0.85 (−4.81, 3.11)	−4.43 (−8.09, −0.77)
Visual matching	−0.49 (−1.61, 0.64)	−0.39 (−2.04, 1.25)	2.13 (−0.14, 4.40)	1.08 (0.03, 2.13)
Numbers reversed	2.61 (−1.07, 6.29)	5.76 (2.04, 9.47)	−2.29 (−10.41, 5.83)	−4.71 (−11.61, 2.19)
GIA score	0.70 (−0.59, 1.99)	1.64 (−0.35, 3.63)	0.99 (−1.16, 3.14)	−1.39 (−3.71, 0.93)
Verbal ability	−0.16 (−1.75, 1.43)	0.94 (−1.30, 3.18)	1.12 (−0.69, 2.93)	0.20 (−3.43, 3.82)
Thinking ability	0.54 (−0.51, 1.58)	1.07 (−0.66, 2.80)	1.20 (−1.20, 3.59)	−1.78 (−3.70, 0.13)
Cognitive efficiency	3.27 (−1.29, 7.83)	2.65 (0.01, 5.28)	0.31 (−4.49, 5.12)	−1.33 (−5.59, 2.93)

*Total urinary As adjusted for specific gravity and then natural log transformed.

[1]adjusted for school clusters at the design stage.

[2]adjusted further for child's sex, BMI for age z scores, serum ferritin, blood lead, hemoglobin, season, test administrator, mother's education, crowding at home, HOME inventory score, parental smoking, source of drinking water, and household possessions.

Table 4

Associations between As exposure* (μg/L) and child performance on the Woodcock-Muñoz cognitive battery – stratified by total folate intake.

	Folate intake ≤ 296.8 μg dfe, n=107 β (95% CI)		Folate intake > 296.8 – ≤ 377.3 μg dfe, n=106 β (95% CI)		Folate intake > 377.3 μg dfe, n=108 β (95% CI)	
	Model 1[1]	Model 2[2]	Model 1[1]	Model 2[2]	Model 1[1]	Model 2[2]
Verbal comprehension	2.36 (0.19, 4.54)	−0.01 (−4.35, 4.33)	−0.25 (−4.31, 3.81)	1.47 (−4.16, 7.11)	−2.49 (−4.28, − 0.69)	−0.53 (−2.56, 1.49)
Visual-auditory learning	2.92 (0.99, 4.85)	0.56 (−1.89, 3.00)	2.02 (−1.55, 5.59)	5.44 (−0.55, 11.43)	−1.59 (−3.21, 0.03)	−1.18 (−2.54, 0.19)
Spatial relations	0.39 (−1.67, 2.44)	−0.75 (−5.85, 4.34)	−0.70 (−2.58, 1.19)	1.24 (−2.52, 5.00)	−0.26 (−1.54, 1.02)	1.19 (−0.08, 2.47)
Sound integration	3.65 (−2.62, 9.93)	3.96 (−2.91, 10.83)	5.17 (−0.38, 10.73)	5.44 (2.38, 8.50)	−0.57 (−6.65, 5.52)	0.54 (−4.66, 5.73)
Concept formation	1.30 (−8.51, 11.12)	−6.27 (−10.84, −1.70)	−2.34 (−7.42, 2.74)	−6.13 (−12.87, 0.62)	−1.38 (−7.50, 4.73)	3.56 (1.56, 5.56)
Visual matching	0.91 (−0.68, 2.51)	−0.29 (−1.46, 0.89)	−0.13 (−2.99, 2.73)	−0.85 (−5.63, 3.93)	−1.24 (−2.22, − 0.26)	−0.24 (−1.42, 0.94)
Numbers reversed	8.12 (2.70, 13.53)	5.54 (1.95, 9.14)	−8.53 (−21.59, 4.54)	−13.06 (−34.77, 8.65)	−4.01 (−7.22, −0.78)	−0.60 (−7.28, 6.08)
GIA score	2.70 (0.63, 4.76)	−0.42 (−2.81, 1.98)	−0.74 (−4.92, 3.44)	−1.02 (−7.71, 5.67)	−1.85 (−3.74, 0.04)	0.36 (−1.26, 1.97)
Verbal ability	2.36 (0.19, 4.54)	−0.01 (−4.35, 4.33)	−0.18 (−4.21, 3.85)	1.47 (−4.16, 7.11)	−2.49 (−4.28, − 0.69)	−0.53 (−2.56, 1.49)
Thinking ability	1.51 (−0.97, 3.98)	−1.73 (−4.20, 0.74)	0.07 (−4.69, 4.84)	0.05 (−4.02, 4.12)	−0.98 (−4.07, 2.11)	1.02 (−0.54, 2.59)
Cognitive efficiency	4.64 (1.33, 7.95)	2.72 (0.55, 4.89)	0.77 (−11.47, 13.01)	−6.34 (−19.91, 7.23)	−2.62 (−4.57, − 0.68)	−0.41 (−4.13, 3.32)

*Total urinary As adjusted for specific gravity and then natural log transformed.

[1]adjusted for school clusters at the design stage.

[2]adjusted further for child's sex, BMI for age z scores, serum ferritin, blood lead, hemoglobin, season, tester, mother's education, crowding at home, HOME inventory score, parental smoking, source of drinking water, and household possessions.