Published in final edited form as:

Environ Int. 2018 February ; 111: 43–51. doi:10.1016/j.envint.2017.11.013.

Nutritional status and diet as predictors of children's lead concentrations in blood and urine

Katarzyna Kordas¹, Rachael Burganowski¹, Aditi Roy², Fabiana Peregalli^{3,4}, Valentina Baccino³, Elizabeth Barcia³, Soledad Mangieri³, Virginia Ocampo³, Nelly Mañay⁵, Gabriela Martínez⁵, Marie Vahter⁶, and Elena I. Queirolo³

¹Epidemiology and Environmental Health, University at Buffalo, Buffalo NY

²Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN

³Centre for Research, Catholic University of Uruguay, Montevideo, Uruguay

⁴Department of Gastroenterology, Hepatology and Nutrition, Pereira Rossell Hospital, Montevideo, Uruguay

⁵Toxicology Area, Faculty of Chemistry, University of the Republic of Uruguay, Montevideo, Uruguay

⁶Institute of Environmental Health, Karolinska Institutet, Stockholm, Sweden

Abstract

Lead exposure remains an important public health problem. Contaminated foods may act as a source of lead exposure, while certain nutrients may reduce lead absorption. We examined the cross-sectional associations of dietary patterns and the intake of several nutrients and foods with blood (Pb-B) and urinary (Pb-U) lead concentrations in children (5–8 y) from Montevideo, Uruguay. From two 24-hour recalls completed by caregivers, we derived the mean daily intake of select nutrients and food groups (dairy, milk, fruit, root vegetables, foods rich in heme and non-heme iron), as well as “nutrient dense” and “processed” food patterns. Pb-B (n=315) was measured using atomic absorption spectrometry; Pb-U (n=321) using ICP-MS. Pb-U was adjusted for specific gravity and log-transformed to approximate a normal distribution. Iron deficiency (ID) and dietary variables were tested as predictors of Pb-B and log-Pb-U in covariate-adjusted regressions. Median [5%, 95%] Pb-B and Pb-U were 3.8 [0.8 – 7.8] µg/dL and 1.9 [0.6 – 5.1] µg/L, respectively; ~25% of Pb-B above current U.S. CDC reference concentration of 5 µg/dL. ID was associated with 0.75 µg/dL higher Pb-B, compared to non-ID (p<0.05). Consumption of root vegetables was not associated with Pb-B or log-Pb-U. Higher scores on the nutrient-dense pattern were related with higher Pb-Bs, possibly due to consumption of green leafy vegetables. Dietary intake of iron or iron-rich foods was not associated with biomarkers of lead. Conversely, children consuming more calcium, dairy, milk and yogurt had lower Pb-B and log-Pb-U. Our findings

Corresponding author: Katarzyna Kordas, Epidemiology and Environmental Health, University at Buffalo, 270 Farber Hall, Buffalo, NY 14214, kkordas@buffalo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

appear consistent with existing recommendations on including calcium-rich, but not iron- or vitamin-C-rich foods in the diets of lead-exposed children, especially where the consumption of these foods is low.

Keywords

blood lead; urinary lead; child; diet; nutrient; Uruguay

1. INTRODUCTION

Childhood exposure to lead, a ubiquitous neurotoxicant, is a significant concern globally (Attina and Trasande 2013; Hanna-Attisha et al. 2016; Laborde et al. 2015; Mitra et al. 2009). Foods are an important source of lead exposure (CONTAM 2010). At the same time, nutritional factors and diet as a whole may modify the gastrointestinal absorption, and possibly, the toxic effects of lead (Kwong et al. 2004; Wright 1999). Yet, the relationship between diet and lead toxicity is not entirely clear. With few exceptions, this research has mostly focused on single nutrients.

Among the most thoroughly studied single nutrients, the deficiency of iron (ID) is associated with higher blood lead concentrations (Pb-Bs) in children, in large part due to increased absorption of lead via the Divalent Metal Transporter 1 (DMT1) (Kordas 2010; Wright et al. 2003). On the other hand, higher dietary iron intake is associated with lower Pb-Bs (Hammad et al. 1996; Schell et al. 2004). Iron fortification appears to hold promise for lowering children's Pb-B (Bouhouch et al. 2016; Zimmermann et al. 2006), but important questions remain regarding this strategy (Kordas 2017). For calcium, while higher dietary intake was associated with lower Pb-Bs in some observational studies (Lacasaña et al. 2000; Mahaffey et al. 1986; Schell et al. 2004), the efficacy of calcium supplementation in lowering children's Pb-Bs has been questioned (Ballew and Bowman 2001; Kordas 2017). Evidence for other nutrients, including vitamin C and zinc, is mixed (Rosado et al. 2006; Schell et al. 2004; Simon 1998).

In contrast to individual nutrients, some of which have been found to impair the intestinal absorption of lead, little evidence is available on which foods, food groups or dietary patterns could effectively prevent lead exposure or lower children's Pb-Bs. This is despite recommendations from the U.S. CDC on the nutritional management of children with elevated Pb-Bs, which includes the daily provision of one serving of red meat, as well as of calcium- and vitamin C-rich foods (CDC 2002b). In one study, the consumption of hamburgers, doughnuts, and peanut butter and jelly sandwiches, was associated with higher Pb-B in 13–24 month-olds (Freeman et al. 1997). In the same study, 13–24 and 25–36 month-olds who consumed yogurt had lower Pb-Bs (Freeman et al. 1997). In a randomized controlled trial, ground fish consumption had no benefit in reducing Pb-Bs in young children compared to placebo (Keating et al. 2011). A further complication in the lead-diet relationship is that foods, including candy (CDC 2002a; Tamayo y Ortiz et al. 2016), and vegetables and cereals (Callan et al. 2014; Chen et al. 2011; Jin et al. 2014), may be an important source of lead exposure.

Given the gaps in understanding of how nutrients and foods impact the exposure to, excretion, and accumulation of lead in children's bodies, particularly at low or very-low level of lead exposure, our objective was to assess the associations between the intake of several nutrients and foods, as well as dietary patterns, with Pb-B and Pb-U in Uruguayan children. We have shown previously that children in this setting are exposed to metals, including lead (Kordas et al. 2010; Queirolo et al. 2010).

2. METHODS

2.1 Study setting and participant recruitment

This cross-sectional study was conducted in Montevideo, the capital of Uruguay, from July 2009 to August 2013. Children who participated in the study attended 11 private elementary schools in several municipal areas where lead exposure was suspected based on clinical experience of one of the co-authors (EIQ) or previously reported in the literature (Queirolo et al. 2010). Leaded gasoline was a major source of lead exposure until it was phased out in 2004; however, lead contamination of dust and soil likely persists and contributes to exposure among children. Additionally, informal battery recycling and occupational exposures of the parents have become more prominent sources.

Participant recruitment has been described in detail previously (Roy et al. 2015). All 673 first-grade children attending the participating schools were eligible, 357 were enrolled. The sole exclusion criterion for the study was a previous diagnosis of Pb-B > 45 µg/dL, a level which would have necessitated medical intervention. None of the children were excluded based on this criterion. The study was approved by ethics committees at the Pennsylvania State University, the Catholic University of Uruguay, and the Faculty of Chemistry at the University of the Republic of Uruguay.

Socio-demographic information—Parents/caregivers who agreed to participate completed a questionnaire about socio-demographic characteristics of the family, the child's medical history and the home environment. They reported their age, education, occupation, smoking history, and family structure. To assess socio-economic status (SES), caregivers provided information on home ownership, number of rooms, number of persons living in the house, and family possession of 12 common household (ex., TV, washer, cellular phone, and car). An SES index was computed from a factor analysis of household assets. A single factor consisting of 5 items was retained. The resultant score (0–5) was split at the median for use in statistical analysis. Household occupant density was calculated based on the number of people living in the house divided by the number of rooms. Crowding was defined as occupant density >2 persons/room.

2.2 Sample collection and lead analysis

Lead concentrations for this study were analyzed in blood and urine. Urinary Pb concentrations (Pb-U) were included as an indicator of lead excretion. As opposed to Pb-B, which has a half-life of months, Pb-U has a half-life of days. Venous blood was collected in the morning (between 8 and 11 am), after an overnight fast by a phlebotomy nurse. Lithium heparin coated trace-metal free tubes (Becton Dickinson) were used for data collection.

Samples were transported on ice to the Toxicology Laboratory in the Faculty of Chemistry at the University of the Republic of Uruguay. Blood lead concentrations were measured 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 flame AAS and 0.7 µg/dL for graphite furnace AAS, respectively. There were no values below the limit of detection. The analytical method was taken into account in statistical analysis. Analytical conditions were validated with standard quality assurance/quality control procedures (Parsons and Chisolm 1997). The laboratory participates in CDC's Lead and Multi-Element Proficiency Program (LAMP) and the Interlaboratory Program of Quality Control for Lead in Blood, Spain (PICC Pb-S).

Children collected first void urine samples and brought them to school on the same morning as the blood draw. Samples were collected in polyethylene cups that had previously been rinsed repeatedly with 10% HNO₃ and deionized water to avoid contamination. Parents received instructions for urine collection at home, including capturing urine mid-stream. The urine samples were transported on ice to the Toxicology Laboratory of the University of the Republic, Montevideo. Specific gravity of each sample was measured using a portable specific gravity refractometer (PAL 10S, Atago Inc, USA) on the day of the collection. Lead concentrations in urine were analyzed in two batches at the Karolinska Institutet, Sweden. Urine was diluted 1:10 with 1% nitric acid (65% w/w, Scharlau, Scharlab S.L., Sentmenat, Spain) and the measurement was performed on an Agilent 7700× ICP-MS (Agilent Technologies, Tokyo, Japan), equipped with collision/reaction cell technology. The limit of detection was 0.005 ng/g in batch 1 and 0.0009 ng/g in batch 2. No batch differences in (Pb-U) were detected. There was one value below the limit of detection and the actual reported value was used in statistical analysis.

We analyzed two reference urine samples (Serorm Urine 1011644 L1 and Serorm Urine 1011645 L2) with recommended concentrations 0.66 and 90.7 µg/L). The obtained average concentrations were 0.51±0.01 and 80.43±0.71 µg/L, respectively (n=4). To compensate for the variation in dilution of the urine samples, Pb-U's were adjusted the average specific gravity (SG, Mean [range]: 1.024 [1.003 – 1.045]). Adjustment by SG is less affected by body size, muscle mass and diet (particularly meat intake), than creatinine adjustment (Nermell et al. 2008).

2.3 Nutritional status assessment

Fasting venous blood was also drawn into a serum tube with clot activator and separator gel (Vacutainer SST Tube, Becton Dickinson). Immediately following the draw, a drop of blood was removed from this tube to measure the child's hemoglobin concentration using a portable hemoglobinometer (HemoCue, Lake Forest, CA). Quality control checks were performed daily using standard HemoCue controls (low, medium, high) provided by the manufacturer. Approximately 45 min after the blood draw, serum was separated by centrifuging for 10 min at 3000 rpm.

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). The ELISA assay was used when the laboratory no longer had the capability to handle radioactive materials. Intra- and inter-assay coefficients (CV) were 4.2% & 9.5% respectively for the IRMA method and 1.7% and 7.6% for the ELISA method; CV below 10% is typically considered acceptable. 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. Serum C-Reactive Protein (CRP) was analyzed in duplicate using an ELISA technique (Erhardt et al. 2004). Serum control samples (Liquicheck, Bio-Rad) were used as standards. Intra-assay and inter-assay CV were 4.9% and 8%, respectively.

Based on the age range of the study children (5 – 8 years) and WHO guidelines (WHO 2001), anemia was defined as hemoglobin less than 11.5 g/L and iron deficiency (ID) as CRP-adjusted serum ferritin of less than 15 ng/mL.

On the day of the blood draw, children were also weighed in triplicate using a digital scale (Seca 872, Shorr Productions, Colombia, MD) and their height was measured in triplicate using a portable stadiometer (Seca 214, Shorr Productions, Colombia, MD). A final weight was calculated by averaging the three measurements and subtracting standard weights of children's clothing. Based on the mean weight (corrected for clothing) and height measurements, each child's Body Mass Index (BMI) was calculated. Z-scores on weight-for-age, height-for-age and BMI-for-age were calculated using the WHO Anthro Plus software (WHO, 2010). Overweight (BMI-z-score > +1 SD) and obesity (BMI-z-score > +2 SD) were defined according to WHO reference points (de Onis et al. 2007).

2.4 Diet assessment and dietary nutrient intakes

To determine children's dietary intake, two 24-hour dietary recalls were conducted by trained nutritionists with the mother or another caregiver familiar with the child's diet. The child was present at the time and contributed to the recall. This is important because caregivers often prepare and pack multiple foods or snacks, but children may not consume all of them. When the caregiver was unaware of or forgot the cafeteria menu, the nutritionist communicated with the school to obtain the details. One recall took place at the school on the day of the blood draw. The second recall took place over the phone without prior appointment, at least 2 weeks later, and fell either on a weekday or a weekend (the median [5%, 95%] time interval between recalls was 19 [13, 47] days and 90% of recalls were conducted within 1 month of one another). Three separate contact attempts were made by telephone to complete the interview.

A detailed list of all the foods and beverages the child consumed within the previous 24-hour period was collected. Information was obtained about the name of the meals, time and place of consumption, amounts of foods consumed or food portions, food preparation methods, recipe ingredients and brand names of commercial products. Food models and household measurements were used during the interviews to facilitate food portion recalls, and to quantify the amount and volume of foods/beverages consumed. Use of vitamin and mineral

supplements, not very common in this population, was also queried. Neutral probing questions such as “Did your child eat/drink anything on the way home from school yesterday?” or “Did he/she have anything before going to bed?” were asked to get accurate dietary information. All foods were assigned a unique code and entered, along with amounts consumed, into a database that contained the nutrient composition of typical Uruguayan foods and preparations (342 unique foods or food preparations such as pizza or noodle soup). The database was created specifically for this study and was based on information provided by Uruguayan food manufacturers or obtained from a number of published resources, including, among others, nutrient composition of Central American (INCAP and OPS 2012) and Spanish (Cruz and Cervera Ral 1986) foods for items common between Uruguay and these regions, as well as recipes from the dietary guidelines for Uruguayans published by the National Diet Institute (INDA 2010). Current mineral fortification laws in Uruguay were taken into consideration. Since 2006, all commercially produced wheat flours in Uruguay are fortified with 2.4 mg of folic acid and 30 mg of elemental iron per kg. The intakes of iron, zinc, vitamin C, folate, and fiber were derived from the database. In addition, proportion of energy from carbohydrate, protein and fat was calculated.

Dietary nutrient intakes from the two 24-hour recalls were averaged, however, where a single recall was collected (2%), that value was used. The consumption of most nutrients is positively correlated with total energy intake (Willet 1998). To account for the fact that individual differences in total energy intake produce variation in intake of specific nutrients unrelated to dietary composition, all nutrient intake values were adjusted for total energy and are expressed as mg or micrograms of nutrient per 1000 kcal/day. Because intakes of most nutrients were not normally distributed, all dietary macro and micronutrients were split into tertiles for further statistical modeling.

2.5 Food groups, dietary and meal patterns

Dietary patterns were constructed as described previously (Kordas et al. 2016) based on 25 food groups (1) fruit & juices, 2) dark leafy vegetables, 3) red and orange vegetables, 4) beans and peas, 5) other vegetables, 6) potatoes, 7) breads, 8) grains, 9) pasta, 10) red meats, 11) white meats, 12) processed meats, 13) eggs, 14) milks, 15) cheeses, 16) yogurt, 17) soy products, 18) fats and oils, 19) milk-based desserts, 20) sweets, 21) pastries, 22) potato chips and fried potatoes, 23) sweetened beverages, 24) pizzas and dinner pies, 25) sauces and condiments) developed from a list of 342 foods present in the database of Uruguayan foods. These food groups were subjected to principal component analysis (PCA) and two food patterns were identified, with specific foods (listed below) having a component loading of at least 0.2: 1) “processed foods”—higher consumption of breads, processed meats, fats and oils, sweetened beverages, and yogurt; reduced intake of milk, pastries and pizza dinners; 2) “nutrient dense”— higher consumption of dark leaf and red-orange vegetables, higher consumption of eggs, beans & peas, potatoes; reduced consumption of pasta and sauces/condiments. The two food pattern scores obtained in PCA analysis were normally distributed and used as continuous variables in statistical models.

We constructed additional food groups to consider the potential of calcium-rich dairy foods (including milk, cheese and yogurt), and heme-iron-rich foods (red and white meats), and

non-heme-iron-rich foods (green beans, peas, lentils, beans, soy/bean burger, spinach, and dried fruit) to contribute to lower Pb-B in children. We also constructed a root-vegetable grouping (carrots, beets, potatoes and sweet potatoes) to test its potential for contributing to lead exposure. Milk and yogurt were also examined separately. The total intake of foods in each food group was expressed on a per-1000 kcal basis to account for differences in caloric intake. The food groups, except for root vegetables, were divided into tertiles. The distribution of root vegetable intakes was highly skewed, with most children reporting no intake. Therefore, a median split was applied, dividing the sample into non-consumers and consumers. Other vegetable sources were not considered due to very low intakes.

2.6 Statistical analyses

The study was based on two dependent variables—Pb-B and log-Pb-U—tested in separate Ordinary Least Square (OLS) regression models. There were four types of independent variables: 1) iron status (ID and anemia); 2) dietary nutrient intake (iron, zinc, calcium, vitamin C, folate, fiber, % energy from protein, carbohydrates and fats); 3) foods (dairy, yogurt, milk, root vegetables, heme- and non-heme-iron-rich foods, fruit); 4) nutrient-dense and processed food patterns. Distributions of all dependent and independent variables were explored. With the exception of dietary patterns, which remained continuous, all independent variables were categorized (ID, anemia, and root vegetables as dichotomous; nutrients and other foods as tertiles) for analysis. Initially, the mean $\pm$ SD or median [range] of lead biomarkers were summarized at each level of the categorical predictor variables. Next, the associations between Pb-B and log-Pb-U and each of the independent variables (above) were tested in separate regression models. Dose-response relationships were also tested for tertiles of nutrient and food group intakes. For dietary pattern variables, where both nutrient dense and processed foods factor scores were modeled together.

All covariates were chosen *a priori* based on previous literature (Schell et al. 2004; Wang et al. 2017) and consisted of (variables are continuous unless otherwise specified): child age, sex (boy/gril), BMI, CRP-adjusted serum ferritin (except in regressions modeling iron status), household possessions score, season (fall, winter, spring, summer), current smoking by either parent (yes/no), crowding in the home (yes/no), the presence of any parental occupational exposure (yes/no), maternal education (any primary, any secondary, any university or higher), maternal occupation (unemployed/stay at home, employed in domestic/janitorial services, job requiring less formal training, and job requiring more formal training), and season of recall (winter/spring/summer/fall). In addition, Pb-B models were adjusted for the analytical method (flame vs. graphite furnace AAS) used to measure lead in blood. We considered the issue of adjusting for multiple comparisons. In our choice of a correction factor, we considered a balance between avoiding false positive results without falsely rejecting true positive findings. Because we conducted 3 families of statistical tests (1=unadjusted; 2=covariate-adjusted; 3=trend tests), corrected our initial p-value for the three families of “comparisons” to arrive at a p-value of <0.02 indicating significance.

3. RESULTS

3.1 Sample Characteristics

The analytical sample for this study consisted of 335 children with at least one Pb biomarker. The mean age of the study children was 6.7 years, and 55.2% were boys (Table 1). Approximately half the mothers of the study children had at least partially completed secondary education, and half being either unemployed or working in domestic or janitorial services. Crowding was present in 22% of the homes.

In terms of nutritional status, 40% of the children were either overweight or obese, while ~8% were underweight and 4% had anemia. After accounting for CRP (marker of inflammation), nearly 40% of children had serum ferritin concentrations <15 ng/mL (Table 1). The children consumed a mean of just under 2,200 ± 560 kcal/day, with more than half of the kilocalories coming from carbohydrates (57%), followed by fat (30%), and protein (13%). Based on U.S.-based Dietary Reference Intakes (IOM 1997, 2001), 52% of children were below the RDA for zinc (5 mg/d), 87% for calcium (1000 mg/d), 29% for vitamin C (25 mg/d), 59% for iron (10 mg/d) and 4% for folate (200 µg/d).

The mean ± SD PB-B of the study sample was 4.2 ± 2.1 µg/dL, with a median [5%, 95%] of 3.8 [0.8 – 7.8] µg/dL; 75 children (23.8%) fell at or above the current U.S. CDC reference concentration of 5 µg/dL. The median [5%, 95%] Pb-U was 1.9 [0.5 – 5.1] µg/L (Table 1). Spearman correlation coefficient for Pb-B and Pb-U was 0.30 (p<0.001).

3.2 Iron status and biomarkers of lead

Children with ID (CRP-adjusted serum ferritin < 15 ng/mL) had higher Pb-B in both unadjusted (β [95% CI]: 0.98 [0.51, 1.45]) and covariate-adjusted 0.75 [0.22, 1.27] models. Non-anemic children had slightly higher Pb-B than children without anemia (0.20 [−0.01, 0.41] and 0.18 [−0.05, 0.42] in unadjusted and covariate-adjusted models, respectively), but this did not reach statistical significance. Neither indicator of iron status was associated with log-Pb-U.

3.3 Micronutrient intakes and lead biomarkers

Estimated iron or folate intake from the daily diet was not associated with either Pb-B or log-Pb-U. Children with the highest tertile of zinc intake had lower Pb-B (−0.63 [−1.21, −0.06]) than children in the lowest tertile (Table 2), but this was attenuated after covariate adjustment. The p-value for trend (p=0.03) in the unadjusted models indicated a dose-response relationship between zinc intake and Pb-B, but this also became attenuated (p=0.10) after covariate adjustment. On the other hand, with covariate adjustment, the association between the highest tertile of dietary zinc and log-Pb-U became slightly stronger (−0.18 [−0.38, 0.03], p=0.09), as did the dose-response relationship (p=0.09 for trend). In covariate-adjusted analyses, children in the highest tertile of calcium intake had lower Pb-B than children in the lowest tertile (−0.51 [−1.11, 0.09], p=0.09). Also in a covariate-adjusted model, there was a dose-response relationship between categories of calcium intake and Pb-B but this did not reach statistical significance (p=0.09). Dietary calcium intake was also related to lower log-Pb-U (unadjusted −0.32 [−0.50, −0.13]; covariate-adjusted −0.31

[-0.52, -0.10]). There was a dose-response relationship with higher calcium intake and log-Pb-U ($p < 0.01$ in both unadjusted and covariate-adjusted models). Given some of the above evidence for the associations between zinc, calcium and iron intake and children's Pb-B and log-Pb-U, we conducted exploratory analyses fitting the tertiles of all three nutrients into a single model. Despite the presence of the other nutrients in the model, the highest tertile of calcium intake remained inversely associated with Pb-B (-0.52 [-1.13, 0.09], $p = 0.095$) and log-Pb-U (-0.28 [-0.50, -0.08], $p = 0.008$).

Children consuming higher proportion of energy from protein had lower lead concentrations in blood and urine in a dose-response manner ($p = 0.02$ for Pb-B and $p = 0.04$ for log-Pb-U for trend in unadjusted models), but these associations became attenuated after covariate adjustment (Online Table 1). No other macronutrient was associated with either Pb-B or log-Pb-U in covariate-adjusted models.

3.4 Food group consumption, dietary patterns, and biomarkers of lead

There was high variability of intakes of food groups: all dairy (median, [range] 433 [0 – 1169.5] g/day, milk 350 [0 – 950] g/d, fruits 100 [0 – 950] g/d, root vegetables 0 [0 – 220] g/d (only 32% reported any root vegetable consumption), heme iron sources 50 [0 – 400] g/d, non-heme iron sources 35 [0 – 255] g/d.

Children consuming highest amounts of all dairy products together had lower Pb-B and log-Pb-U, but only for Pb-U was this association statistically significant (Table 3). Children falling into the highest tertile of milk consumption had lower log-Pb-U in both unadjusted and covariate-adjusted models. The estimated associations for dairy and milk were very similar, suggesting that the dairy-urinary lead association was mostly driven by milk consumption. Dose-response relationships were observed between tertiles of all dairy ($p < 0.01$) and milk ($p < 0.01$) consumption and log-Pb-U, but not Pb-B, in covariate-adjusted models. Further exploration of covariate-adjusted models of dairy foods revealed lower Pb-B in children falling in the medium (-0.79 [-1.60, 0.01]) and highest tertile (-0.70 [-1.22, -0.18]) of yogurt consumption, compared to lowest tertile. A dose-response relationship was also observed between yogurt consumption tertiles and Pb-B ($p < 0.01$).

Because dairy products can be a source of both protein and calcium, we explored the extent to which these nutrients explain the association between dairy consumption and Pb-B or log-Pb-U. We fit three additional covariate-adjusted models by including 1) dietary calcium intake, 2) proportion of energy consumed as protein, and 3) both calcium and protein to the “dairy” model. This additional adjustment made no difference to the previously reported dairy-Pb-B associations. For log-Pb-U, the beta coefficients for dairy (reported from Table 2 — β [95% CI] for tertile 2: -0.08 [-0.28, 0.13; tertile 3: -0.27 [-0.47, -0.07]) became attenuated and non-significant when dietary calcium was entered into the models (tertile 2: -0.2 [-0.23, 0.20]; tertile 3: -0.12 [-0.36, 0.13]). Adding protein into the model had no effect on the beta coefficients for dairy (tertile 2: -0.08 [-0.28, 0.13]; tertile 3: -0.27 [-0.48, -0.07]). Adding both calcium and protein intake resulted in inferences similar to those produced by dairy & calcium intake.

There were no associations between fruit or root vegetable consumption and either Pb-B or log-Pb-U (Table 3). Because fruit consumption is an important source of vitamin C, we carried out an additional model, that included both fruit and vitamin C. Adding vitamin C to “fruit” models did not change the inferences in any way (data not shown).

Similarly, neither the consumption of heme nor non-heme iron sources was associated with biomarkers of lead (Table 3), even when we adjusted for dietary vitamin C, an important promoter of iron absorption (data not shown). The two dietary patterns identified in PCA were modeled together in a single model because they were uncorrelated following orthogonal rotation and, together, were deemed to represent the totality of the child’s food consumption. The processed food pattern was not associated with either Pb-B or log-Pb-U, but higher consumption of nutrient-dense foods was associated with slightly higher Pb-B, even after adjusting for covariates (Table 3).

4. DISCUSSION

This cross-sectional study of school-aged urban children in Uruguay, 25% of whom had Pb-B $\geq 5\mu\text{g/dL}$ (the current U.S. CDC reference concentration), confirmed that children with iron deficiency (low serum ferritin) had higher Pb-B than children who were not deficient. The study expands current knowledge of lead-diet interactions by demonstrating that: 1) estimated higher calcium, milk, and overall dairy intakes were associated with lower log-Pb-U; associations with Pb-B were in the anticipated direction but not statistically significant; 2) the intake of calcium appears to explain most of the association between milk/dairy consumption and lower urinary Pb concentrations; 3) intake of dietary iron or of heme or non-heme iron-rich foods was not associated with Pb-B or log-Pb-U despite clear links with iron status; 4) fruit and root vegetable consumption was not related to lead biomarkers; 5) vitamin C intake was not associated with either Pb-B or Pb-U; 6) nutrient-dense dietary pattern was associated with slightly higher Pb-B.

Lead is absorbed in the small intestine, via tight junctions between epithelial cells (Holt et al. 1987) and nutrient-specific transporters (Ballew and Bowman 2001; Bannon et al. 2002). Several factors may affect the absorption, including lead solubility, the presence of dietary components (Holt et al. 1987), as well as underlying iron status. For example, in the GI tract lead forms stable insoluble ligands with anions such as carbonate and phosphate, which in part explains why less lead is absorbed in the fed versus fasted state (Holt et al. 1987). Iron and lead also appear to compete for absorption by iron transporters (Bannon et al. 2002). Both children and adults who have ID have been found to have higher Pb-B than individuals without ID, likely due to increased absorption of lead by DMT1 (Kordas 2010); our findings are consistent with this literature.

With respect to dietary intake of iron, we found no association with biomarkers of lead, despite ~40% of children having low iron stores (serum ferritin $< 15\text{ ng/mL}$). Some previous observational studies conducted among infants and preschoolers showed inverse associations (Hammad et al. 1996; Schell et al. 2004), and two of three iron-supplementation or fortification trials conducted to date were efficacious in lowering Pb-B in school-age children (Bouhouch et al. 2016; Zimmermann et al. 2006). Although, Uruguayans generally

consume large amounts of meat, 60% of the children in our study did not meet the recommended daily iron intakes of 10 mg/day. This contrasts with the study of 9–12 month-old infants from Albany, 20% of whom were below the RDA (Schell et al. 2004). Furthermore, half the children in our study ate an average of less than 1 meat serving per day (50 g, 1.8 oz). The consumption of non-animal sources of iron (for example, spinach, beans, raisins and products made with iron-fortified flour) was low, with half the children not consuming any of these foods.

According to the recommended management of children with elevated Pb-B, the CDC includes the daily provision of one serving (2–3 oz) of red meat (CDC 2002b). Meat is an excellent source of heme iron; and meat consumption improves children's iron stores (Szymlek-Gay et al. 2009). Our study is the first to investigate not only the association between the intake of iron-rich foods and children's Pb-B, but also to separate heme and non-heme iron-rich foods. Neither of these groupings was associated with differences in lead biomarkers. Importantly, higher meat consumption (heme iron) was also not related to serum ferritin concentrations or the likelihood of having ID. Additional studies are needed to investigate these relationships more thoroughly.

We found no association between lead biomarkers and the intake of vitamin C. For the management of elevated Pb-B, the CDC recommends that children consume a diet rich in sources of vitamin C (CDC 2002b), in part because vitamin C ought to contribute to improved iron status of lead-exposed children. Few other studies have investigated these associations to compare our findings. Among 6–16 year-olds participating in NHANES III, higher serum ascorbic acid concentrations were associated with lower prevalence of Pb-B >15 µg/dL (Simon and Hudes 1999). In the same study, however, dietary intake of vitamin C was not related to the prevalence of elevated Pb-B (Simon and Hudes 1999). We also examined the link between fruit consumption and the biomarkers of lead. Children in the highest tertile of fruit consumption had 20 mg/1000 kcal higher vitamin C intake than children in the lowest tertile. On a per-weight basis, the most commonly consumed fruits in this study were apples > bananas > oranges = fruit salad > fruit juices. These fruits are a good, but not excellent (except for oranges), source of vitamin C. This fact, combined with generally low intakes (median consumption of any fruit was 0 g), may partly explain the null associations. Finally, we included both fruit consumption and vitamin C intake into a single model, and found that this strategy was no more informative than the fruit or the vitamin C model alone. Additional studies are needed to clarify these relationships.

Calcium is another dietary factor of interest with respect to lead exposure. Very little work thus far has related the intake of calcium-rich foods and children's Pb-B. In our study, children consumed relatively low amounts of calcium, and over 60% had daily intakes below the U.S. Institute of Medicine recommendation of 800 mg. In this low-intake context, possibly representing a calcium-deficient population, children with highest calcium consumption (386 – 812 mg/kcal/day) had lower Pb-B ($p<0.10$) and Pb-U ($p<0.05$) than children with lowest consumption (51 – 279 mg/1000 kcal/day). Our study is consistent with previous observational evidence on calcium intake (Lacasaña et al. 2000; Mahaffey et al. 1986; Schell et al. 2004; Turgeon O'Brien et al. 2014), but contradicts findings from supplementation trials, which show limited success in lowering children's Pb-Bs by

providing calcium supplements (Keating et al. 2011; Markowitz et al. 2004) or calcium-rich foods (Keating et al. 2011).

The average daily consumption of milk and dairy products was variable in our study, but 50% of the children consumed 350 g of milk (one glass of milk is 250 ml, roughly 250 g) and 400 g of dairy (less than two cups). Higher milk and dairy consumption were both associated with lower log-Pb-U. Although associations with Pb-B were not statistically significant, they were in the expected direction, and therefore, overall consistent. Furthermore, higher yogurt consumption was associated with lower Pb-B in these children. It is unclear why Pb-B was inversely associated with yogurt consumption and log-Pb-U with milk and overall dairy consumption. As biomarkers, Pb-B and Pb-U are correlated but represent different pools of lead, with different half-lives of elimination from the body (months for Pb-B, days/weeks for Pb-U). Because of these differences in these biomarkers, complete agreement between them or the effects of diet on their metabolism, cannot be expected. Nevertheless, our study agrees with previous work, although evidence is generally scarce. For example, among Mexican women with environmental exposure (mean maternal Pb-B 11.2 µg/dL, mean cord lead concentration 10.8 µg/dL), higher milk, but not yogurt or cheese, intake was associated with lower biomarkers of lead (Hernandez-Avila et al. 1997). Furthermore, young U.S. children (12–36 mo, mean Pb-B 10.7 ± 4.9 µg/dL) who were regular yogurt consumers had ~3 µg/dL lower Pb-B than children who did not consume yogurt (Freeman et al. 1997). More recently, Kim and colleagues also showed an inverse association between the servings of milk and dairy consumed and Pb-B of Korean adolescent girls (geometric mean Pb-B 1.08 µg/dL) (Kim et al. 2017). Because dairy products are the main source of calcium, some involvement of calcium as a mechanism behind our findings is plausible. In fact, higher calcium (but not protein) intake largely explained the association we saw between dairy consumption and urinary lead concentrations.

Specific foods, through food crop cultivation, thoroughness of cleaning prior to consumption, or cooking practices, may also contribute to lead exposure. Because lead remains in the environment for long periods of time, it poses a risk for food contamination; several reports indicated contamination of a variety of fruits and vegetables by lead (Amin et al. 2013; Säumel et al. 2012; von Hoffen and Säumel 2014). When we examined the relationship between the consumption of any root vegetables (carrots, beets, potatoes and sweet potatoes) and children's biomarkers of lead, we found no associations. It is important to note that to construct the root vegetable food grouping we only considered those vegetables that were consumed as discrete dishes, not in mixed preparations such as soups. In considering mixed preparations, we would have been unable to separate the potential effects of the vegetables on biomarkers of lead from the influence of other constituents of the mixed dish. However, our analytical decision could have influenced our findings—root vegetables were not commonly consumed as standalone dishes (half the children reported eating no vegetables over the two days of recall and the highest reported intake was of 200 g or 7 oz, equivalent to ~1 cup of potatoes). It appears that root vegetables do not contribute to increased lead exposure of Uruguayan children, but this should be confirmed in future studies.

In contrast, we found a positive association between the nutrient-dense food pattern and children's Pb-B. This pattern represented higher consumption of dark leafy and red-orange vegetables, as well as eggs, beans/peas, and potatoes, and reduced consumption of pastas and sauces/condiments. Leafy vegetables could be considered high accumulators of lead (Alexander et al. 2006). Tomatoes, a red-orange vegetable in our pattern, have also been shown to be contaminated by lead (Amin et al. 2013). No other studies have investigated dietary patterns in relation to children's biomarkers of lead. Among Korean adults, highest scores on a balanced food pattern were associated with lower likelihood of elevated Pb-B (Chung et al. 2013), whereas among elderly men in the U.S., a Western food pattern was associated with higher bone lead concentrations (Wang et al. 2017). Additional research of this kind is needed and our current findings with respect to dietary patterns need be interpreted with caution, particularly because vegetable-rich diets have strong health benefits, and because we did not ask parents about food preparation (ex. cleaning) practices.

Our findings should be considered in the context of the strengths and limitations of the study. First, only ~50% of families eligible to participate chose to enroll in the study. We cannot speak to differences between participants and non-participants, but of the families that enrolled, 90% provided information on diet and biological samples to measure biomarkers of lead exposure. Second, the study was cross-sectional, and therefore, cannot address the causality of the observed associations. Third, dietary intake was based on caregiver recall over two days. This design is similar to the U.S. NHANES and we included non-consecutive days separated by at least 2 weeks to ensure better representation of typical intakes. We tried to limit measurement error by providing aids to estimate serving sizes, asking about snacking, and asking the child, as well as the school to help recall school meals. We adjusted our statistical models for total caloric intake to reduce the influence of the overall amount of food consumed on the observed associations. We also took account of the variability in consumption of seasonally-available fruits and vegetables by including season of recall as one of the covariates. It is important to consider that the diet constitutes only one lead exposure source; others include water, dust and soil. We do not have information on lead concentrations in dust or soil in the children's homes. By measuring only diet, our study provides a limited picture of the totality of exposures in this population. While we collected data on the source of water for cooking and drinking, we have no way of establishing the pattern of consumption or more importantly, the "dose" of exposure—how much Pb was consumed from water. The amounts of water used for cooking are particularly difficult to measure with recall methods used by our study. Therefore, our study had limited ability to eliminate potential residual confounding of lead exposure from a number of environmental sources. Furthermore, nutritional status and dietary components are just two of the many factors, including gene polymorphisms, that influence metal absorption, metabolism, and final Pb-B or U-Pb measured in a given child. On the other hand, the strength of our study consists of: 1) policy-relevant questions, 2) hypothesis testing based on biological plausibility, 3) inclusion of two biomarkers of lead exposure (Pb-B with half-time of 2–3 months), and 4) thorough control for potential covariates to reduce the possibility of residual confounding. By merging nutrient- and food-based analyses, our study addresses the difficulty of conducting *translatable* research on diet-toxicant interactions. If only information on nutrients is used, the resulting inferences are of limited utility for informing

dietary behaviors because most parents and children are not familiar with the nutrient content of specific foods.

5. CONCLUSION

Our study showed that the consumption of calcium-rich, but not iron-rich, foods is associated with lower blood and urinary concentrations of school-age children. On the other hand, foods consumed by some Uruguayan children, particularly those that form part of a nutrient-dense diet (ex. green leafy or orange vegetables), may constitute a major source of exposure to lead among children, possibly due to improper washing. Further research on the contribution of specific foods to increased lead exposure in this population is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the U.S. National Institute of Environmental Health Sciences (NIEHS) and Fogarty International (FI) Institute under grant R21ES16523, and by NIEHS under grant R21ES019949.

References

- Alexander PD, Alloway BJ, Dourado AM. Genotypic variations in the accumulation of cd, cu, pb and zn exhibited by six commonly grown vegetables. *Environ Pollution*. 2006; 144:736–745.
- Amin N, Hussain A, Alamzeb S, Begum S. Accumulation of heavy metals in edible parts of vegetables irrigated with waste water and their daily intake to adults and children, district mardan, pakistan. *Food Chem*. 2013; 136:1515–1523. [PubMed: 23194556]
- Attina TM, Trasande L. Economic costs of childhood lead exposure in low- and middle-income countries. *Environ Health Perspect*. 2013; 121:1097–1102. [PubMed: 23797342]
- Ballew C, Bowman B. Recommending calcium to reduce lead toxicity in children: A critical review. *Nutr Rev*. 2001; 59:71–79. [PubMed: 11330624]
- Bannon DI, Portnoy ME, Olivi L, Lees PS, Culotta VC, Bressler JP. Uptake of lead and iron by divalent metal transporter 1 in yeast and mammalian cells. *Biochem Biophys Res Comm*. 2002; 295:978–984. [PubMed: 12127992]
- Bouhouch RR, El-Fadeli S, Andersson M, Aboussad A, Chabaa L, Zeder C, et al. Effects of wheat-flour biscuits fortified with iron and edta, alone and in combination, on blood lead concentration, iron status, and cognition in children: A double-blind randomized controlled trial. *Am J Clin Nutr*. 2016; 104:1318–1326. [PubMed: 27733396]
- Callan A, Hinwood A, Devine A. Metals in commonly eaten groceries in western australia: A market basket survey and dietary assessment. *Food Additives Contam*. 2014; 31:1968–1981.
- CDC. Childhood lead poisoning associated with tamarind candy and folk remedies—california, 1999–2000. *MMWR Morb Mortal Wkly Rep*. 2002a; 51:684–686. [PubMed: 12233910]
- CDC. Managing elevated blood lead levels among young children: Recommendations from the advisory committee on childhood lead poisoning prevention. Atlanta, GA: Centers for Disease Control and Prevention; 2002b.
- Chen C, Qian Y, Chen Q, Li C. Assessment of daily intake of toxic elements due to consumption of vegetables, fruits, meat, and seafood by inhabitants of xiamen, china. *J Food Sci*. 2011; 76
- Chung HK, Park JY, Cho Y, Shin MJ. Contribution of dietary patterns to blood heavy metal concentrations in korean adults: Findings from the fifth korea national health and nutrition examination survey 2010. *Food Chem Toxicol*. 2013; 62:645–652. [PubMed: 24120902]
- CONTAM EPoCitFCC. Scientific opinion on lead in food. *EFSA J*. 2010; 8

- Cruz AJ, Cervera Ral P. Tabla de composición de alimentos. 1986
- de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a who growth reference for school-aged children and adolescents. *Bull WHO*. 2007; 85:660–667. [PubMed: 18026621]
- Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and c-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. *J Nutr*. 2004; 134:3127–3132. [PubMed: 15514286]
- Freeman NC, Ettinger A, Berry M, Rhoads G. Hygiene- and food-related behaviors associated with blood lead levels of young children from lead-contaminated homes. *J Expo Anal Environ Epidemiol*. 1997; 7:103–118. [PubMed: 9076612]
- Hammad TA, Sexton M, Langenberg P. Relationship between blood lead and dietary iron intake in preschool children. A cross-sectional study. *Annals of Epidemiology*. 1996; 6:30–33. [PubMed: 8680621]
- Hanna-Attisha M, LaChance J, Sadler RC, Schnepf AC. Elevated blood lead levels in children associated with the flint drinking water crisis: A spatial analysis of risk and public health response. *Am J Public Health*. 2016; 106:283–290. [PubMed: 26691115]
- Hernandez-Avila M, Sanin LH, Romieu I, Palazuelos E, Tapia-Conyer R, Olaiz G, et al. Higher milk intake during pregnancy is associated with lower maternal and umbilical cord lead levels in postpartum women. *Environ Res*. 1997; 74:116–121. [PubMed: 9339224]
- Holt, AC., Partridge, S., Morton, AP., Blair, JA. The intestinal absorption of lead: The importance of lead speciation. In: Lindberg, SE., Hutchinson, TC., editors. *Proceedings of the Heavy metals in the environment: international conference, 1987; New Orleans, LA. Oak Ridge National Laboratory*; p. 375-377.
- INCAP IdNdCAyP, OPS OPdIS. Tabla de composición de alimentos de centroamérica. 2012
- INDA INDA. Manual de alimentación para las familias uruguayas. 2010
- IOM IoM. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin d, and fluoride. Washington DC: National Academy Press; 1997.
- IOM IoM. Dietary reference intakes for vitamin a, vitamin k, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press; 2001.
- Jin Y, Liu P, Sun J, Wang C, Min J, Zhang Y, et al. Dietary exposure and risk assessment to lead of the population of jiangsu province, china. *Food Additives Contam*. 2014; 31:1187–1195.
- Keating EM, Fischer PR, Pettifor JM, Pfitzner M, Isichei CO, Thacher TD. The effect of calcium supplementation on blood lead levels in nigerian children. *J Pediatr*. 2011; 159:845–850. [PubMed: 21665223]
- Kim J, Shin M, Kim S, Seo J, Yang YJ. Association of iron status and food intake with blood heavy metal concentrations in korean adolescent girls and women: Based on the 2010–11 korea national health and nutrition examination survey. *J Nur Health*. 2017; 50:350–360.
- Kordas K. Iron, lead, and children's behavior and cognition. *Annu Rev Nutr*. 2010; 30:123–148. [PubMed: 20433349]
- Kordas K, Queirolo EI, Ettinger AS, Wright RO, Stoltzfus RJ. Prevalence and predictors of exposure to multiple metals in preschool children from montevideo, uruguay. *Sci Tot Environ*. 2010; 408:4488–4494.
- Kordas K, Queirolo EI, Mañay N, Peregalli F, Hsiao PY, Lu Y, et al. Low-level arsenic exposure: Nutritional and dietary predictors in first-grade uruguayan children. *Environ Res*. 2016; 147:16–23. [PubMed: 26828624]
- Kordas K. The “lead diet”: Can dietary approaches prevent or treat lead exposure? *J Pediatr*. 2017 [Epub ahead of print].
- Kwong WT, Friello P, Semba RD. Interactions between iron deficiency and lead poisoning: Epidemiology and pathogenesis. *Sci Tot Environ*. 2004; 330:21–37.
- Laborde A, Tomasina F, Bianchi F, Bruné MN, Buka I, Comba P, et al. Children's health in latin america: The influence of environmental exposures. *Environ Health Perspect*. 2015; 123:201–209. [PubMed: 25499717]

- Lacasaña M, Romieu I, Sanin LH, Palazuelos E, Hernandez-Avila M. Blood lead levels and calcium intake in mexico city children under five years of age. *Int J Environ Health Res.* 2000; 10:331–340. [PubMed: 11260781]
- Mahaffey KR, Gartside PS, Glueck CJ. Blood lead levels and dietary calcium intake in 1- to 11-year-old children: The second national health and nutrition examination survey, 1976 to 1980. *Pediatrics.* 1986; 78:257–262. [PubMed: 3488536]
- Markowitz ME, Sinnott M, Rosen JF. A randomized trial of calcium supplementation for childhood lead poisoning. *Pediatrics.* 2004; 113:e34–e39. [PubMed: 14702492]
- Mitra AK, Haque A, Islam M, Bashar SAMK. Lead poisoning: An alarming public health problem in bangladesh. *Int J Environ Res Public Health.* 2009; 6:84–95. [PubMed: 19440271]
- Nermell B, Lindberg AL, Rahman M, Berglund M, Persson LA, El Arifeen S, et al. Urinary arsenic concentration adjustment factors and malnutrition. *Environ Res.* 2008; 106:212–218. [PubMed: 17900556]
- Parsons, PJ., Chisolm, JJJ. The lead laboratory (Screening young children for lead poisoning: guidance for state and local health officials). Atlanta, GA: Centers for Disease Control and Prevention; 1997.
- Queirolo EI, Ettinger AS, Stoltzfus RJ, Kordas K. Association of anemia, child and family characteristics with elevated blood lead concentrations in preschool children from montevideo, uruguay. *Arch Environ Occup Health.* 2010; 65:94–100. [PubMed: 20439228]
- Rosado JL, Lopez P, Kordas K, Garcia-Vargas G, Ronquillo D, Alatorre J, et al. Iron and/or zinc supplementation did not reduce blood lead concentrations in children in a randomized, placebo-controlled trial. *J Nutr.* 2006; 136:2378–2383. [PubMed: 16920858]
- Roy A, Queirolo E, Peregalli F, Mañay N, Martínez G, Kordas K. Association of blood lead levels with urinary f₂-8α isoprostane and 8-hydroxy-2-deoxy-guanosine concentrations in first-grade uruguayan children. *Environ Res.* 2015; 140:127–135. [PubMed: 25863186]
- Säumel I, Kotsyuk I, Hölscher M, Lenkerei C, Weber F, Kowarik I. How healthy is urban horticulture in high traffic areas? Trace metal concentrations in vegetable crops from plantings within inner city neighborhoods of berlin, germany. *Environ Pollution.* 2012; 165:124–132.
- Schell LM, Denham M, Stark AD, Ravenscroft J, Parsons P, Schulte E. Relationship between blood lead concentration and dietary intakes of infants from 3 to 12 months of age. *Environ Res.* 2004; 96:264–273. [PubMed: 15364593]
- Simon JA, Hudes ES. Relationship of ascorbic acid to blood lead levels. *JAMA.* 1999; 281:2289–2293. [PubMed: 10386552]
- Simon SL. Soil ingestion by humans: A review of history, data, and etiology with application to risk assessment of radioactively contaminated soil. *Health Phys.* 1998; 74:647–672. [PubMed: 9600298]
- Szymlek-Gay EA, Ferguson EL, Heath ALM, Gray AR, Gibson RS. Food-based strategies improve iron status in toddlers: A randomized controlled trial. *Am J Clin Nutr.* 2009; 90:1541–1551. [PubMed: 19828711]
- Tamayo y Ortiz M, Téllez-Rojo MM, Hu H, Hernández-Ávila M, Wright R, Amarasiriwardena C, et al. Lead in candy consumed and blood lead levels of children living in mexico city. *Environ Res.* 2016; 147:497–502. [PubMed: 26974363]
- Turgeon O'Brien H, Gagné D, Vaissière E, Blanchet R, Lauzière J, Vézina C, et al. Effect of dietary calcium intake on lead exposure in inuit children attending childcare centers in nunavik. *Int J Environ Health Res.* 2014; 24:482–495. [PubMed: 24382151]
- von Hoffen LP, Säumel I. Orchards for edible cities: Cadmium and lead content in nuts, berries, pome and stone fruits harvested within the inner city neighborhoods in berlin, germany. *Ecotoxicol Environ Safety.* 2014; 101:233–239. [PubMed: 24507151]
- Wang X, Ding N, Tucker KL, Weisskopf MG, Sparrow D, Hu H, et al. A western diet pattern is associated with higher concentrations of blood and bone lead among middle-aged and elderly men. *J Nutr.* 2017 [Epub ahead of print].
- WHO. Iron deficiency anaemia: Assessment, prevention, and control. Geneva, Switzerland: United Nations Children's Fund, United Nations University, World Health Organization; 2001.

- Willet, W. Issues in analysis and presentation of dietary data. In: Willet, W., editor. Nutritional epidemiology, Part. 2nd. New York: Oxford University Press; 1998.
- Wright RO. The role of iron therapy in childhood plumbism. *Curr Opin Pediatr.* 1999; 11:255–258. [PubMed: 10349106]
- Wright RO, Tsaih SW, Schwartz J, Wright RJ, Hu H. Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. *J Pediatr.* 2003; 142:9–14. [PubMed: 12520247]
- Zimmermann MB, Muthayya S, Moretti D, Kurpad A, Hurrell RF. Iron fortification reduces blood lead levels in children in bangalore, india. *Pediatr.* 2006; 117:2014–2021.

HIGHLIGHTS

- Links between children's nutritional status, diet and biomarkers of lead were assessed.
- Iron deficiency was associated with higher blood lead concentrations.
- Higher consumption of calcium-rich foods like milk and yogurt was linked with lower lead concentrations in blood and urine.
- Higher consumption of iron-rich or vitamin-C rich foods was not related to blood or urinary lead.

Table 1

Characteristics of children from Montevideo, Uruguay, enrolled in the study.

Characteristic	N	% missing values	Mean $\pm$ SD or %	Median (5%, 95%)
Age (months)	335	0%	81.1 $\pm$ 6.4	81.0 (72.0 – 92.0)
Sex	335	0%		
Male			55.2%	
Female			44.8%	
Either parent currently smokes	302	9.8%	53.3%	
Mother's education	327	2.4%		
Some primary			19.9%	
Some secondary			54.4%	
College or higher			26.7%	
Mother's occupation	317	5.4%		
Unemployed/stay at home			30.9%	
Domestic/janitorial services			18.9%	
Jobs requiring additional training			50.2%	
Parents have potential occupation exposure to metals	335	0%	26.9%	
Household is crowded ¹	302	9.8%	21.5%	
Household possessions score below median of 4 items	305	9.0%	54.7%	
Source of household drinking water	300	10%		
Unfiltered tap/tank			31.3%	
Filtered tap			19.0%	
Bottled, with other sources			49.7%	
Blood lead concentration ($\mu\text{g}/\text{dL}$)	315	6.0%	4.2 $\pm$ 2.1	3.8 (0.8 – 7.8)
Urinary lead concentration ($\mu\text{g}/\text{L}$) ²	325	3.0%	2.3 $\pm$ 1.8	1.9 (0.6 – 5.1)
Body Mass Index (kg/m^2)	325	3.0%	16.9 $\pm$ 2.6	16.4 (13.8 – 22.3)
Overweight or obese ³			39.9%	
Hemoglobin (g/dL)	321	4.2%	13.2 $\pm$ 1.1	13.1 (11.6 – 15.2)
Anemia ($<11.5 \text{ g}/\text{dL}$)			4.0%	
Serum ferritin (ng/mL) ⁴	303	9.5%	20.2 $\pm$ 14.0	18.0 (3.1 – 45.2)
Iron deficient ($<15 \text{ ng}/\text{mL}$)			39.6%	

¹ Crowding defined as more than 2 persons per bedroom living in the house;² Concentration adjusted for specific gravity of urine to account for individual variability in hydration level;³ Overweight or obese defined as BMI-for-age Z-score > 1 SD;

⁴ Adjusted for C-reactive protein to account for variability due to inflammation or infection.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2

Associations among the intakes of micronutrients (iron, zinc, calcium, vitamin C and folate), and biomarkers of lead exposure among school-age children from Montevideo, Uruguay.

Nutrient intake (mg or µg/1000 kcal/day)	Blood lead concentration β [95% CI]		Urinary lead concentration ¹ β [95% CI]	
	Unadjusted	Covariate-adjusted ²	Unadjusted	Covariate-adjusted ²
Iron (Fe)				
Low (1.98 – 3.91)	Ref.	Ref.	Ref.	Ref.
Medium (3.92 – 4.88)	0.22 [–0.36, 0.80]	0.43 [–0.16, 1.03]	0.01 [–0.17, 0.20]	–0.05 [–0.26, 0.15]
High (4.89 – 49.38)	–0.21 [–0.80, 0.37]	–0.05 [–0.66, 0.56]	–0.11 [–0.29, 0.08]	–0.10 [–0.31, 0.11]
p-value for trend test	0.47	0.87	0.26	0.34
Zinc (Zn)				
Low (0.48 – 1.79)	Ref.	Ref.	Ref.	Ref.
Medium (1.81 – 2.75)	–0.39 [–0.96, 0.19]	–0.39 [–0.98, 0.20]	–0.01 [–0.20, 0.17]	0.10 [–0.19, 0.21]
High (2.77 – 6.91)	–0.63 [–1.21, –0.06]**	–0.50 [–1.10, 0.10]	–0.14 [–0.32, 0.04]	–0.18 [–0.38, 0.03]*
p-value for trend test	0.03	0.10	0.13	0.09
Calcium (Ca)				
Low (50.68 – 275.50)	Ref.	Ref.	Ref.	Ref.
Medium (276.10– 383.95)	–0.23 [–0.81, 0.34]	–0.42 [–1.01, 0.17]	–0.09 [–0.27, 0.07]	–0.14 [–0.34, 0.06]
High (384.16– 811.84)	–0.48 [–1.06, 0.10]	–0.51 [–1.11, 0.09]*	–0.32 [–0.50, –0.13]**	–0.31 [–0.52, –0.10]**
p-value for trend test	0.10	0.09	<0.01	<0.01
Vitamin C				
Low (0 – 13.11)	Ref.	Ref.	Ref.	Ref.
Medium (13.24 – 24.66)	0.51 [–0.07, 1.08]*	0.53 [–0.08, 1.14]*	0.07 [–0.11, 0.26]	0.10 [–0.11, 0.31]
High (24.85 – 137.34)	0.43 [–0.15, 1.01]	0.36 [–0.24, 0.97]	0.01 [–0.18, 0.19]	0.07 [–0.14, 0.28]
p-value for trend test	0.14	0.25	0.95	0.53
Folate				
Low (75.03 – 189.37)	Ref.	Ref.	Ref.	Ref.
Medium (189.78 – 247.49)	0.18 [–0.40, 0.77]	0.50 [–0.12, 1.13]	0.06 [–0.13, 0.24]	0.12 [–0.09, 0.34]
High (249.33 – 466.51)	0.05 [–0.53, 0.63]	0.19 [–0.42, 0.80]	0.09 [–0.10, 0.27]	0.13 [–0.08, 0.34]
p-value for trend test	0.87	0.61	0.36	0.26

¹Urinary lead concentration adjusted for specific gravity of urine to account for individual variability in hydration levels and subsequently log-transformed;

²Models adjusted for child sex, age, BMI, serum ferritin concentration, laboratory method for lead analysis (flame vs. graphite atomic absorption spectrometry), parental smoking status, crowding in the home, parental occupation with potential exposure to metals, maternal education level, maternal occupation, household possessions score, and season of recall.

Table 3

Associations between food consumption and the biomarkers of lead exposure among school-age children from Montevideo, Uruguay.

Food group (g/1000 kcal/day)	Blood lead concentration B [95% CI]		Urinary lead concentration ^I B [95% CI]	
	Unadjusted	Covariate-adjusted ²	Unadjusted	Covariate-adjusted ²
Dairy				
Low (0.00 – 158.76)	Ref.	Ref.	Ref.	Ref.
Medium (159.12 – 233.41)	0.23 [–0.34, 0.81]	0.17 [–0.44, 0.77]	–0.02 [–0.20, 0.16]	–0.08 [–0.28, 0.13]
High (234.11 – 692.48)	–0.23 [–0.80, 0.34]	–0.18 [–0.77, 0.42]	–0.25 [–0.43, –0.07]**	–0.27 [–0.47, –0.07]**
p-value for trend test	0.45	0.56	0.007	0.009
Milk				
Low (0.00 – 105.07)	Ref.	Ref.	Ref.	Ref.
Medium (106.88 – 197.55)	0.20 [–0.38, 0.77]	0.14 [–0.46, 0.74]	–0.08 [–0.26, 0.11]	–0.08 [–0.28, 0.13]
High (198.13 – 499.98)	–0.12 [–0.70, 0.46]	0.06 [–0.54, 0.66]	–0.25 [–0.43, –0.07]**	–0.27 [–0.47, –0.07]**
p-value for trend test	0.70	0.83	0.007	0.009
Fruits				
Low (0.00 – 28.44)	Ref.	Ref.	Ref.	Ref.
Medium (28.75 – 70.29)	0.57 [–0.01, 1.14]*	0.23 [–0.37, 0.84]	0.09 [–0.09, 0.28]	–0.14 [–0.06, 0.35]
High (70.93 – 302.63)	0.44 [–0.13, 1.02]	0.10 [–0.50, 0.69]	–0.02 [–0.20, 0.16]	0.01 [–0.20, 0.21]
p-value for trend test	0.13	0.77	0.82	0.97
Root vegetables				
None	Ref.	Ref.	Ref.	Ref.
Any (3.13 – 105.07)	–0.20 [–0.70, 0.31]	–0.18 [–0.71, 0.35]	–0.08 [–0.24, 0.08]	–0.07 [–0.25, 0.11]
Foods rich in heme iron				
Low (0.00 – 11.95)	Ref.	Ref.	Ref.	Ref.
Medium (12.34 – 38.94)	–0.04 [–0.62, 0.54]	0.18 [–0.42, 0.79]	–0.12 [–0.31, 0.06]	–0.17 [–0.38, 0.03]
High (39.87 – 207.22)	0.10 [–0.48, 0.68]	0.28 [–0.32, 0.88]	–0.09 [–0.28, 0.09]	–0.08 [–0.28, 0.13]
p-value for trend test	0.74	0.35	0.33	0.49
Foods rich in non-heme iron				
Low (0.00 – 9.71)	Ref.	Ref.	Ref.	Ref.
Medium (9.72 – 25.94)	0.20 [–0.38, 0.78]	0.37 [–0.23, 0.97]	0.0 [–0.18, 0.19]	–0.04 [–0.24, 0.17]
High (25.97 – 133.36)	0.03 [–0.55, 0.61]	0.07 [–0.54, 0.69]	–0.05 [–0.23, 0.14]	–0.09 [–0.30, 0.12]
p-value for trend test	0.93	0.82	0.61	0.42
Dietary patterns³				
Processed score	0.01 [–0.16, 0.17]	–0.01 [–0.17, 0.16]	0.04 [–0.01, 0.09]	0.03 [–0.02, 0.09]
Nutrient-dense score	0.20 [0.03, 0.37]**	0.19 [0.01, 0.37]**	0.01 [–0.05, 0.06]	0.01 [–0.05, 0.07]

¹Urinary lead concentration adjusted for specific gravity of urine to account for individual variability in hydration levels and subsequently log-transformed;

²Models adjusted for child sex, age, BMI, serum ferritin concentration, laboratory method for lead analysis (flame vs. graphite atomic absorption spectrometry), parental smoking status, crowding in the home, parental occupation with potential exposure to metals, maternal education level, maternal occupation, household possessions score, and season of recall;

³The dietary pattern analysis modeled both pattern scores together in a single model.