Published in final edited form as:

Sci Total Environ. 2019 March 25; 658: 1239–1248. doi:10.1016/j.scitotenv.2018.12.220.

A cross-sectional study of urinary cadmium concentrations in relation to dietary intakes in Uruguayan school children

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Abstract

Background: Cadmium (Cd) exposure has adverse health effects in children. Diet contributes to Cd exposure, but dietary components could affect body Cd levels.

Objective: To examine associations between diet and urinary Cd (U-Cd) in children.

Methods: In this cross-sectional study conducted in Montevideo, Uruguay, Cd exposure of 5-8 year old children (n=279 with complete data) was assessed in first morning urine (U-Cd), a marker of long-term exposure, measured by ICP-MS and adjusted for specific gravity. Distribution of U-Cd was (median [5%, 95%]: 0.06 [0.02, 0.17] µg/L); data were natural-log-transformed (ln) for statistical analyses. Serum ferritin (SF), an indicator of iron stores, was measured in fasting samples. Trained nutritionists completed two non-consecutive 24-hr dietary recalls with both child and caregiver present. Measures of iron, zinc, calcium and fiber intake, and the consumption of grains, root vegetables, milk, and foods rich in heme iron (white and red meats) and non-heme iron (legumes, spinach, broccoli, tomatoes, dried fruit) were derived. Multivariable ordinary least squares (OLS) and ordinal regressions were used to examine associations among tertiles of water Cd, SF, diet, and U-Cd. OLS models were further stratified by sex.

Results: In covariate-adjusted models, SF was not related to ln-U-Cd. Children in highest tertile of iron and zinc intake had lower ln-U-Cd: (−0.23 [−0.42, −0.03]) and (−0.25 [−0.44, −0.05], respectively, compared to the reference group. Children consuming higher amounts of foods rich

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in heme iron had slightly lower ln-U-Cd [-0.17 ($-0.36, 0.03$)]. High grain consumption was related to higher ln-U-Cd (0.25 [$0.06, 0.45$]).

Conclusions: Diets rich in grains were related to higher urinary Cd levels among children living in the context of low Cd pollution. Higher intake of iron and zinc was related to lower Cd levels. Given that urinary Cd is mainly a marker of long-term exposure, these findings should be further corroborated.

GRAPHICAL ABSTRACT

Keywords

Urinary cadmium; diet; child; grains; iron; zinc

1. INTRODUCTION

Exposure to cadmium (Cd) among children has been associated with impaired growth (Gardner et al., 2013; Kippler et al., 2012; Lin et al., 2011) and neurodevelopmental deficits (Gustin et al., 2018; Kippler et al., 2016; Liu et al., 2015). The primary routes of Cd exposure relevant for young children are food and contaminated soil (more likely ingested by crawling infants and toddlers) (CDC, 2009). Diet is the main source of Cd exposure in the general non-smoking population (EFSA, 2009; Satarug et al., 2017). Elevated Cd concentrations have been found in cereals, particularly rice, as well as leafy green vegetables and root vegetables (CDC, 2009; Kippler et al., 2010; Zhong et al., 2015); and the consumption of diets rich in cereals and vegetables is associated with higher intake of Cd (Fagerstedt et al., 2015; Krajcovicová-Kudláčková et al., 2006). Seafood, including crustaceans and mollusks, may contain high Cd concentrations (Mendil et al., 2010; Olmedo et al., 2013; Storelli et al., 2012). Offal and sausage, which frequently contain liver and kidney meats and can be a major source of Cd, have been related with higher blood Cd levels (Friedman et al., 2006; Madeddu et al., 2011; Roca et al., 2016). Generally, drinking water contributes negligibly to population-level exposure (Rango et al., 2015; Riederer et al., 2013; Zailina et al., 2014), but it may be an additional source in people living close to a contaminated site (Wang et al 2015).

The link between the diet and body levels of Cd is fairly complex. As mentioned above, food can be a source of Cd exposure, thus resulting in higher Cd levels in blood or urine. Diet is also a source of nutrients that, when ingested, could lower the absorption of Cd through direct competition for intestinal transporters (for example, iron, zinc) or by forming

insoluble complexes (for example, fiber) that are not absorbed. In addition, low iron status may increase intestinal Cd absorption. Several studies, mostly conducted in adults, have reported higher Cd in blood or urine in individuals with low vs. replete iron status (Akkermans et al., 2016; Barany et al., 2005; Berglund et al., 1994; Madeddu et al., 2011; Shah et al., 2011; Turgut et al., 2007). This happens because low iron stores result in up-regulated expression of the divalent metal transporter (DMT1) to absorb more iron from the diet. DMT1 has also a high affinity for Cd, thus resulting in higher blood Cd levels (Akesson et al., 2002; Berglund et al., 1994).

Across the globe, children well into school age, are at risk of developing micronutrient (for example, iron, zinc) deficiencies (Poh et al., 2013; Rojroongwasinkul et al., 2013; Sandjaja et al., 2013). Nevertheless, few studies exist to aid in understanding how diets variable in the content of these nutrients may affect biomarkers of Cd in children. This question is important in children because of apparently higher rates of Cd accumulation in the kidneys compared to adults (Kippler et al., 2010). In one study, 24-hr recalls were collected among children, adolescents and adults participating in the U.S. NHANES. Dietary iron was inversely associated with urinary Cd, but only among smoking adults (Riederer et al., 2013). In the same study, calcium intake was also not associated with urinary Cd among children. On the other hand, there is some evidence that both calcium (Madeddu et al., 2011; Suarez-Ortegón et al., 2013) and zinc (Suarez-Ortegón et al., 2013) are negatively associated with blood Cd among adult females.

Given the paucity of data on the relation between diet and Cd body burdens in children, our main objectives were to evaluate the relationship between urinary Cd (U-Cd) concentrations, and iron status and diet in a cross-sectional study of 6-8 y old children living in Montevideo, Uruguay. Specifically, we investigated the following dietary components: 1) dietary intake of iron, zinc and fiber, 2) consumption of diets with variable iron content, and 3) consumption of potential food sources of Cd. The inclusion of foods rather than just nutrients, and the distinction between foods rich in heme vs. non-heme iron may provide more translatable information on the relationship between diet and body burdens of Cd than studies to date.

2. METHODS

2.1. Study Overview

This study utilized participant data from a cross-sectional school-based study conducted in Montevideo, Uruguay between July 2009 and August 2013. The participating schools were chosen because of their location in areas of Montevideo with previously documented exposure to metals (Kordas et al., 2010; Manay et al., 2008). Participants were first grade children (age 5-8 years old) attending private elementary schools within several neighborhoods of Montevideo. Of the 673 eligible participants, 53% (N=357) were enrolled into the study with parental consent. The study was approved by the Institutional Review Boards or Ethics Committees at the Catholic University of Uruguay, Ethics Committee of Faculty of Chemistry University of the Republic of Uruguay, Pennsylvania State University, and the University at Buffalo.

As described previously (Kordas et al 2016, Kordas et al, 2017), study recruitment occurred through a two-step process, with school directors being invited first; once the director's approval was granted, meetings for parents were held to inform them about study procedures and obtain consent. To be enrolled, children had to be attending first grade on a regular basis and have no history of blood lead level (BLL) $\geq 45 \mu\text{g/dL}$, which would have necessitated medical intervention. No children were excluded based on this criterion, partly because very few parents were aware of their children's BLLs. If siblings participated in the study, the first sibling enrolled was removed from analysis ($n=4$), to conserve independence of observations.

2.2. Specimen Collection

2.2.1. Blood Collection and Analysis—A phlebotomy nurse collected fasting venous blood at the child's school between the hours of 8 am and 11 am. A portable Hemocue hemoglobinometer (HemoCue Inc. Lake Forest, CA) was used to measure hemoglobin concentration using a few drops of venous blood. Quality control tests were performed daily. Hemoglobin below 11.5 g/L was defined as anemia (WHO, 2001).

Serum ferritin (SF) was analyzed in duplicate using at first an immunoradiometric assay (Coat-A-Count Ferritin IRMA; SIEMENS Diagnostic Products, USA). The laboratory then switched to an enzyme immunoassay (Spectro Ferritin, RAMCO Laboratories, Texas, USA) when it could no longer process radioactive materials. The intra- and inter-assay coefficients were 4.2% and 9.5% for the IRMA method and 1.7% and 7.6% for the ELISA method. Using the IRMA method as the gold standard, a correction factor was established for the ELISA-based results. CRP, a marker of inflammation, was analyzed using an ELISA technique and established protocols (Erhardt et al., 2004). Serum control samples were used as standards (Liquicheck, Bio-Rad). The intra and inter-assay coefficients of variation were 4.9% and 8%, respectively. CRP-adjusted SF values below 15 ng/mL were considered to indicate iron deficiency, ID (WHO, 2001).

2.2.2. Urine Collection and Analysis—Children were given acid-washed [10% HNO_3 and deionized H_2O] 100 mL sample cups, which they used to provide a first morning urine sample. These sample cups were closed tightly with their corresponding lids, and brought to school. Samples were kept on ice at the school, and then stored at -20°C at the Catholic University. U-Cd were measured at Karolinska Institutet, Sweden, as described previously (Kippler et al., 2007; Kippler et al., 2010). The urine was diluted [1:10 with 1% nitric acid] (65% w/w, Scharlau, Scharlab S.L., Sentmenat, Spain) and analysis was done using an Agilent 7700x ICP-MS (Agilent Technologies, Tokyo, Japan), equipped with octopole collision/reaction cell technology to minimize spectral interferences (Kippler et al., 2007). The samples were analyzed in two batches, with a limit of detection of $0.0005 \mu\text{g/L}$ for batch 1 and $0.001 \mu\text{g/L}$ for batch 2; there were no batch differences. One value reported by the laboratory was below the LOD, but it was used in analysis as given. Urinary Cd concentrations were adjusted for specific gravity (SG) of urine using the formula ($\text{U-Cd} \cdot [\text{SG}_{\text{mean}} - 1] / [\text{SG}_i - 1]$), where “mean” refers to the mean specific gravity of the study sample (0.024) and “i” refers to each individual. Specific gravity was measured on the day

of collection using a portable specific gravity refractometer (PAL 10S, Atago Inc, USA), to account for variation in urine dilution.

It is important to consider the choice of this biomarker vis-à-vis the study's purpose of estimating dietary predictors of Cd levels in children's bodies. While blood Cd represents more recent exposure (months), urine is used to examine long-term Cd exposure and accumulation in the kidneys (Jarup et al., 1998; Kippler et al., 2010; Tchounwou et al., 2012; Wittman and Hu, 2002). With a long half-life in the kidneys (6-38 years) and liver (4-19 years), and due to the bioaccumulation of cadmium in these tissues, urinary Cd estimates longer-term exposure and is usually proportional to levels found in the kidneys (ATSDR, 1999; Vacchi-Suzzi et al., 2016). However, due to the young age of the study children, it is likely that their kidneys have probably not accumulated much cadmium, and for this reason, urinary Cd probably also reflects ongoing exposure. In a recent study in 5-9 years old Bangladeshi children from Bangladesh, the correlation between blood and urinary Cd was 0.51 (Vahter, unpublished study).

2.2.3. Water Collection and Analysis—Water, which according to the caregiver was used for drinking or cooking, was collected in a 100 ml plastic cup directly from the kitchen water taps or from a water storage container according to established methodology (Carreón Valencia, López Carillo, Romieu 1995). Approximately 15 ml of water was taken from the collection cup by a syringe (Becton-Dickinson, Franklin Lake, NJ, USA) and passed through a 0.45 µm filter (VWR International, PA, USA) into a plastic bottle, previously rinsed with 10% nitric acid and deionized water. The water's pH was measured using an indicator strip and 1-2 drops of concentrated nitric acid (63%) were added to adjust the water pH to less than 2. The water samples were immediately transferred to the Research Center, Catholic University of Uruguay and stored at 4°C until their shipment to the Pennsylvania State University within 6 months of collection.

For the determination of water Cd concentrations, samples at the Material Characterization Laboratory of the Pennsylvania State University by inductively Coupled Plasma Mass Spectrometry (ICP-MS) with Collision Cell Technology (Thermo Scientific XSERIES 2, Bremen, Germany) as described in detail previously (Kordas et al 2016). Briefly, samples were digested with concentrated HNO₃ (~70%, ultra-pure grade) dried at 80°C. Dried samples were dissolved in 2% HNO₃ (ultra-pure grade) to reconstitute the original sample volume. Trace elements in water (NIST 1643) were used for analysis as the instrument standard. The detection limit of was 0.01-0.1 ng/ml. 20.5% of values were reported by the laboratory as being below the LOD; to use them in statistical analyses, we calculated LOD/2.

2.2.4. Dietary Assessment—As described in detail elsewhere (Kordas et al., 2017; Kordas et al., 2016), trained nutritionists conducted two 24-hr recalls of the children's diets, with caregivers as informants. The recalls were spaced at least two weeks apart, with median gap of 19 days. Children were present during these recalls, as a way to minimize errors, especially for the school hours. When the caregiver and/or child could not recall the school lunch menu, the nutritionist consulted with lunch room staff. The first recall occurred at the child's school and the second at least two weeks later, over the phone. The nutritionists

asked caregivers to provide detailed information on the foods and beverages consumed by the child, including food name, time, place, amount/portion, food preparation methods, ingredients and brands of foods. Aids and neutral probing questions were used to facilitate proper recall. These questions included if the child ate or drank anything during certain times of the day and during various activities. The foods were entered into a on database of nutrient composition of 342 individual foods commonly consumed by Uruguayan children, and created specifically for this study (Kordas et al., 2017). The database accounted for current mineral fortification laws, which include the fortification of all commercially-produced wheat flour with 30 mg elemental iron and 2.4 mg of folic acid per kilogram of flour. Total iron, zinc and fiber intake was calculated for each 24-hr recall separately, and then averaged. Nutrient intakes were standardized by total energy consumed and expressed per 1000 kcals/day (Willett et al., 1997).

To create food groups of interest (foods rich in heme and non-heme iron, milk, grains, and root vegetables), the averaged 24-hr dietary recalls (grams of food/day) were used, with individual foods included if they were known known examples of the larger group and if non-zero responses were recorded (at least some children reported consuming the item). Based on known sources of heme iron, red and white meats were grouped together. Red meats consisted of consumption of whole cuts of beef, lamb and pork, ground meat, hamburgers, offal, ribs, tripe, chorizo, whereas white meats consisted of whole chicken, chicken breast, wings, drumsticks, game meats, fresh fish prepared whole, fish fillets, tuna and other canned fish, frozen breaded fishes and prepared foods with fish. The meats group did not include coldcuts. In turn, based on known sources of non-heme iron, this food group included green beans, peas, lentils, beans, soy/bean burger, spinach, broccoli, tomatoes, and dried fruit. The milk group represented milks with different fat content (from whole to skim), milk fortified with vitamins and iron and cream.

The grain (white and whole wheat flour, corn flour and starch, cereals, rice, granola and cereal bars, muesli, polenta and prepared dishes containing rice or grains) and root vegetable (potatoes, French fries, sweet potatoes, carrots, beet, turnips) groupings were created to represent potential food sources of Cd. Leafy green vegetables were not considered in this analysis due to very low consumption (only 12.9%) of the sample had reported any consumption over the two days of recall. All food groups were adjusted for each child's kilocalorie consumption, and expressed per 1000 kcals/day (Willett et al., 1997).

2.2.5. Anthropometry—At the school and between the hours of 8 and 11 am, the child's weight and height were taken in triplicate using a digital scale (Seca 872, Shorr Productions, Columbia, MD) and a portable stadiometer (Seca 214, Shorr Productions, Columbia, MD), respectively. The three measures were averaged, after adjusting for clothing each child wore. Body mass index (BMI) was calculated.

2.3. Statistical Analysis

All statistical analyses were conducted using STATA (version 14.0). Participants lacking the urinary cadmium indicator were excluded (n=28), as were those missing relevant covariates (n=33) or diet information (n=13), leaving a complete-case sample of 279 children.

Two types of models were used to examine the association between nutritional and dietary variables and urinary Cd: 1) Ordinary Least Square (OLS) models and 2) ordinal regression models. For OLS, urinary Cd were first adjusted for specific gravity of urine and then natural-log-transformed to approximate a normal distribution. For ordinal regressions, urinary Cd adjusted for specific gravity was converted into a 4-level categorical variable, with cut points representing the quartiles of the distribution. Log-transformation allows the highly skewed U-Cd variable to have a normal distribution, but transformation compromises interpretability of the regression estimates, given that beta coefficients represent mean change in the outcome that now has a very different scale from the original variable. Therefore, we capitalized on ordinal regression modeling, which used the untransformed distribution of U-Cd split into quartiles, to examine the association between dietary predictors and the likelihood of being in progressively higher categories of U-Cd. The two approaches are meant to corroborate one another.

Several variables were considered for inclusion in the final, covariate-adjusted models: age, BMI, season of study (summer, fall, winter, spring), crowding at home (yes/no), potential for parental occupational exposure to metals (yes/no), maternal education (years), maternal employment status (yes/no), and household possessions score. The final set of covariates was determined through the construction of directed acyclic graphs (DAGs) and consisted of age, maternal education, and household possessions and crowding to represent socioeconomic status. DAGs are visual representations of potential causal relationships among exposure, outcome variables, and related predictor variables, and are used to identify and select confounders into regression models (Shrier and Platt, 2008).

To determine whether water Cd concentrations should be included as a predictor in regression models, we tested the association Cd concentrations in drinking water (W-Cd) to urinary Cd concentrations. We constructed unadjusted and multivariable OLS and ordinal regression models with tertiles of W-Cd as the independent variable.

To investigate the association between nutritional status, dietary factors and U-Cd, the predictor variables were split into tertiles (serum ferritin concentration, dietary iron, zinc and fiber intake, consumption of foods rich in heme iron, grains) or dichotomized (foods rich in non-heme iron, root vegetables). Separate OLS and ordinal regressions were fit for each nutritional /dietary factor, first unadjusted and then adjusted for covariates. For all categorical variables, the lowest group was used as reference.

2.3.1. Secondary Analyses—We performed a number of secondary analyses. First, the intake of many nutrients from the same diet may be correlated. To understand if any nutrients were associated with ln-U-Cd independently of the others, dietary iron, zinc, calcium and fiber intake were entered into a single model; due to the limited sample size, just the trend effects were tested. Similarly, because food consumption is correlated with nutrient intake, for those foods that were associated with ln-U-Cd, we modeled the food and the nutrients it contains in a single model to investigate potential mediation effects. Finally, we stratified the OLS analyses described above by sex to investigate potential differences between boys and girls.

3. RESULTS

3.1. Sample Characteristics

The study sample consisted of 279 children (56% boys, 44% girls) with a mean age of 6.7 years (81 months, range 71-105, Table 1). Just over half of the children had at least one parent who smoked. This was a relatively low-income sample, with over half of the mothers either working in domestic/janitorial services (20%) or unemployed (32%), roughly half of the mothers having some secondary education and about a quarter of the households considered crowded (>2 people per bedroom). Of the children enrolled in the study, 39% were overweight/obese. Anemia was not prevalent in this sample (4%) but 38.7% of children with available data had low iron stores (CRP-adjusted serum ferritin <15 ng/mL). Children consumed ~2,200 kcal/day, with a range of 940-4240 kcal/day. The consumption of the dietary categories of interest was generally low, as follows (median, [5%, 95%]: dietary iron 9.5 [4.9, 19.1] mg/d; dietary zinc 4.8 [0.9, 14.1] mg/d; dietary calcium 710 [300, 1200] mg/d; dietary fiber 3.0 [1.3, 17.7] g/d; foods rich in heme iron 50 [0, 190] g/d; foods rich in non-heme iron 0 [0, 50] g/d; grains 18 [0, 134] g; root vegetables 0 [0, 150] g/d; milk 350 [0, 650] g/d.

The studied children also had relatively low U-Cd: 0.06 [0.02, 0.17] µg/L (Table 1), although four children had U-Cd of 0.27 – 0.42 µg/L and one child had U-Cd of 1.06 µg/L. Online Figure 1 presents a comparison of U-Cd of children from the present study to those observed in children of similar ages living in other settings. Only 191 of the 279 children included in the study had water Cd measures available, with median [5%, 95%] of values falling at 0.03 [0.01, 0.26] µg/L.

3.2. Main Effects Analyses

Because all models were conducted with natural-log-transformed U-Cd values, the beta coefficients should be interpreted with reference to the distribution of those values: mean ± SD (range) -2.84 ± 0.68 (−4.94, 0.05). Higher child age was associated with higher urinary Cd: each 1 month of age was related to β [95%CI] of 0.01 [0.003, 0.03] in ln-U-Cd. Parental smoking, on the other hand was not associated with urinary Cd among children: 0.07 [−0.09, 0.23]. The household W-Cd was not associated either with ln-U-Cd or with the proportional odds of having elevated U-Cd (Table 2). Because of these null associations and a potential reduction in sample size (from 279 to 191) if W-Cd were to be used as a predictor of U-Cd in remaining models, we chose to exclude this variable from further analyses.

The serum ferritin concentration, an indicator of iron stores, was not associated with ln-U-Cd. The findings were as follows: in covariate-adjusted models, compared to tertile 1 (1.2-12.8 ng/mL) of SF, children in tertile 2 (12.9-24.1) and tertile 3 (24.2-95.6) did not differ in terms of ln-transformed U-Cd (0.01 [−0.20, 0.21] and −0.09 [−0.30, 0.12] respectively) or the proportional odds of elevated U-Cd (1.08 [0.62, 1.87] and 0.92 [0.52, 1.62], respectively). Figures 1 and 2 represent covariate-adjusted associations between quantiles of dietary intakes and ln-U-Cd and the proportional odds of elevated U-Cd, respectively. Online Table 1 shows unadjusted associations from both OLS and ordinal regression models. In terms of the dietary variables related to iron intake, children in the

highest tertile of dietary iron intake had lower ln-U-Cd compared to the low-intake reference group (β [95% CI]: -0.23 [$-0.42, -0.03$] in covariate-adjusted model, Figure 1). This is equivalent to one-third of the SD of the ln-U-Cd distribution or 8% of the mean value. The p-value for trend ($p < 0.001$) suggested a dose-response relationship. On the other hand, the intake of iron was not associated with proportional odds of moving across the tertiles of U-Cd (Figure 2). The highest tertile of zinc intake (-0.25 [$-0.43, -0.05$]) and both levels of fiber intake (tertile 2: -0.18 [$-0.38, 0.01$] and tertile 3: -0.17 [$-0.37, 0.02$] $p < 0.1$) were associated with lower ln-U-Cd. For the higher zinc intake, ordinal regression results also suggested ~50% lower odds of children moving into higher U-Cd categories ($p < 0.01$, Figure 2). The intake of calcium was not associated with ln-U-Cd (Figure 1) or with the odds of having elevated U-Cd (Figure 2).

The consumption of foods rich in heme iron (red and white meats) was modestly related to lower ln-U-Cd, (-0.17 [$-0.36, 0.03$] in the covariate-adjusted model; $p = 0.09$). Children in the highest tertile of meat consumption also had 40% lower proportional odds of moving across U-Cd categories compared to the reference group ($p < 0.1$, Figure 2). The consumption of non-heme food sources of iron (beans, peas, lentils, soy/bean burgers, spinach and dried fruit, iron fortified breads/pasta) was not associated with either ln-U-Cd or the proportional odds of elevated U-Cd. The consumption of milk was unassociated with ln-U-Cd (Figure 1) but children in the highest tertile of consumption had 37% lower odds of being in a higher U-Cd category ($p < 0.1$, Figure 2).

Estimated daily consumption of grains or grain-products was associated with higher ln-U-Cd in a dose-related manner ($p = 0.01$) and children in the highest tertile of grains had higher ln-U-Cd compared to the low-intake reference group (0.25 [$0.06, 0.45$] in the covariate-adjusted model, Figure 2). In the context of the ln-U-Cd distribution, children consuming higher amounts of grain had Cd concentrations that were ~8% higher than the reference group. Results from ordinal regression models were consistent with these findings (Figure 2). Finally, any consumption of root vegetables was unrelated with ln-U-Cd or odds of elevated U-Cd.

3.3. Secondary Analyses

The intake of the nutrients from the same diet/foods may be correlated. The dietary intake of nutrients in this study had the following correlations (Spearman rho): iron-zinc, 0.30 ($p < 0.01$), iron-fiber 0.41 ($p < 0.01$), zinc-fiber 0.01 ($p = 0.90$), iron-calcium 0.30 ($p < 0.01$), zinc-calcium 0.15 ($p = 0.01$), fiber-calcium 0.09 ($p = 0.13$). To understand if any nutrients were associated with ln-U-Cd independently of the others, all were entered into a single covariate-adjusted model; due to the limited sample size, just the trend effects were tested. In covariate-adjusted models, only the association between zinc intake and ln-U-Cd remained (-0.11 [$-0.21, -0.01$]).

The associations between foods rich in heme iron and ln-U-Cd observed above may be mediated by the nutrients these foods contain. When dietary iron and zinc intake were entered into the same covariate-adjusted model as foods rich in heme iron, the overall iron intake was associated with lower ln-U-Cd (-0.09 [$-0.19, 0.01$], $p = 0.08$) whereas the other

two dietary factors were not, suggesting that the association of meat consumption with urinary Cd is due to a large extent to the iron content of those foods.

Generally, stratification by sex yielded findings that were consistent with the main-effects models (Online Table 2), with the 95% CIs largely overlapping between the sexes.

4. DISCUSSION

This cross-sectional study of 5-8 y-old children from Montevideo, Uruguay, indicates fairly low-level chronic cadmium exposure. Still, we found that: 1) children consuming grains had higher urinary Cd concentrations; 2) the intake of micronutrients, particularly zinc, was associated with lower U-Cd; 3) children consuming more foods rich in heme iron had somewhat lower U-Cd; 4) the consumption of non-heme food sources was not associated with U-Cd; 5) iron stores were not associated with U-Cd. These findings are discussed below.

Cd exposure among first-graders from Montevideo was low.

The median specific-gravity-adjusted urinary cadmium concentration was 0.06 µg/L (5%, 95%: 0.02, 0.17 µg/L). This is similar to levels reported for U.S. school children participating in the 1999-2004 NHANES (median 0.078 for 6-7 y-olds (Ciesielski et al., 2012), higher than in Danish children (median 0.021 µg/L), but lower than in children from several European countries (median ranges 0.14 – 0.38 µg/L) (Aguilera et al., 2010; Exley et al., 2015; Fucic et al., 2015; Molina-Villalba et al., 2015; Protano et al., 2016; Roca et al., 2016) or Mexico (median 0.14 µg/L) (Moynihan et al., 2017) and especially lower than in children with rice-based diets, for example, 0.30 µg/L in 5 year-old rural Bangladeshi children (Kippler et al., 2010). We found no difference in urinary cadmium between girls and boys. Similarly, there were no sex differences in urinary cadmium in the 2004-2009 NHANES (Riederer et al., 2013).

Children consuming higher amounts of grains had higher U-Cd.

This finding was consistent between the two analytical approaches we employed. Cereals, as well as vegetables have been shown to contain elevated amounts of Cd (CDC, 2009; Zhong et al., 2015), although, for any given population group the greatest impact on dietary exposure to Cd will come from the foods most commonly consumed—in South-East Asia that might be rice, in other populations it could be grains, vegetables or starchy tubers (EFSA, 2012). Few other studies have ascertained the relationship between diet and biomarkers of Cd exposure in children. Among Spanish children, higher consumption of vegetables but not cereals was related to higher urinary Cd (Pérez et al., 2018). We did not find an association with root vegetables (potatoes, carrots, beets) and were unable to test for associations with leafy vegetables due to very low consumption.

Micronutrients, particularly zinc, were associated with lower U-Cd.

This finding was observed in OLS for iron and fiber, but was less obvious in the ordinal regression models. For zinc, both models pointed to a strong association with lower U-Cd levels. It is important to point out that the intake of iron and zinc was modestly correlated

(rho 0.30), as was the intake of iron and fiber (rho 0.41), and iron and calcium (rho 0.30). When all nutrients were entered into a single model, the association with dietary zinc remained, suggesting that it is inappropriate to consider each nutrient alone because of shared food sources. There is little previous research on nutrient intakes from the diet and body cadmium levels in children, to which our findings could be compared. One study in the U.S. did not observe an association between dietary iron, zinc or calcium intake (fiber not examined) and urinary Cd among children (6-11 y) participating in the 1999-2008 cycles of the NHANES (Riederer et al., 2013). In the same study, among adolescents (12-19 y), higher zinc intake was associated with lower urinary Cd levels. The U-Cd concentrations in U.S. children were similar to those observed among Uruguayan children (median 0.06 µg/L). The differing associations by age are worth further investigation because age is related to intestinal absorption and organ retention of Cd (Engström and Nordberg, 1979).

Children consuming more foods rich in heme iron had somewhat lower urinary Cd.

The purpose of conducting a foods-based analysis was to move beyond nutrient intakes alone, and to provide evidence on food sources of particular nutrients in relation to urinary Cd. If appropriate, such evidence may facilitate the translation of findings into recommendations for the public. We divided iron-rich foods into those rich in heme iron and those rich in non-heme iron to reflect the difference in absorption mechanisms—like Cd, non-heme iron is absorbed mostly through the DMT1 mechanism, while heme iron absorption proceeds through a separate, more efficient mechanism (Donovan et al., 2006; Shayeghi et al., 2005). The two types of iron differ in bioavailability (non-heme iron being hindered by the complexes it forms with other food components or the presence of inhibitors such as phytates, tannins, zinc or calcium) and absorption rates (5% vs. 15-35%) (Donovan et al., 2006; Shayeghi et al., 2005).

The association between higher consumption of foods rich in heme iron and U-Cd was marginal ($p < 0.1$) both linear and ordinal regression models, so our findings should be interpreted with caution. Together, the two analytical approaches provide some suggestion that meat consumption may be related to lower urinary Cd. In secondary analyses, we also modeled foods rich in heme iron (meats) consumption together with variables representing total dietary iron and zinc, and found that the association with meats was attenuated while the association with dietary iron remained, at $p < 0.1$. This suggests that the association between foods rich in heme iron and U-Cd is mediated by dietary iron. Interestingly, among Korean adolescent girls, the consumption of meats/eggs and fish/shellfish was not associated with the blood Cd concentration (Kim et al., 2017). We defined foods rich in heme iron as any red and white meats and fish, including whole cuts, ground meat, hamburgers, offal, ribs, tripe. The children ate a median [5%, 95%] of 50 [0, 190] g or 1.7 [0, 6.7] oz of these foods per day, boys slightly more than girls. Recommendations for U.S. children 4-8 years of age include daily consumption of ~4 oz of protein (USDA., 2015).

It is possible that the consumption of meat in the study children displaced grain consumption; meaning that the lower cadmium concentrations could be attributable to lower consumption of grains by children who consume more meats. We attempted to get at this question in post hoc analyses. Grain and heme rich food consumption was uncorrelated

(Spearman $\rho < 0.1$). About 12% of children were in the lowest tertile for the consumption of both grains and meats while another 10% were in the highest tertile for both. We also modeled the tertiles of heme-rich foods and grain consumption together and found exactly the same coefficients and odds ratios in these models as those containing single food groups (refer to Figures 1 & 2), suggesting no displacement. Finally, the consumption of foods rich in heme iron was modestly correlated ($\rho = 0.14$, $p = 0.02$) with children's hemoglobin concentrations (uncorrelated with serum ferritin). Although we did not observe associations between iron status and U-Cd, it is still possible those with better iron status may experience lower Cd absorption.

In contrast, we found no association between the consumption of non-heme rich foods and urinary Cd.

This lack of association could be attributed to overall low consumption of non-heme iron rich foods. The foods making up this group were consumed by less than 10% of the children. This, combined with a low bioavailability of non-heme iron, likely reduced our ability to observe associations if they truly exist. As divalent metals, both iron and Cd use the divalent metal transporter (DMT1) to cross the gut epithelium (Donovan et al., 2006; Fleming et al., 1997; Gruenheid et al., 1995; Gunshin et al., 1997). Non-heme iron comes from plants and iron-fortified foods (NIT, 2016). To create that food category, we combined beans, peas, lentils, soy/bean burgers, spinach, dried fruit, broccoli, and tomatoes. We considered adding to this group iron-fortified breads and pasta, but decided against this because these foods would also fall into the rubric of grains, a potential source of Cd. We reasoned that by excluding grain-based foods we would avoid confounding. Still, we tested for correlations between the consumption on foods rich in non-heme and grains, and found they were uncorrelated ($\rho = 0.05$, $p = 0.37$).

Iron stores were not related to urinary cadmium concentrations.

To counteract a low supply of iron from the diet and the development of iron deficiency (ID), the expression of the DMT1 is upregulated (Ryu et al., 2004). Paradoxically, this also allows for increased absorption of cadmium (Ryu et al., 2004). Previous studies have found higher urinary or blood Cd among children with anemia or ID (Shah et al., 2011; Silver et al., 2013; Turgut et al., 2007). Almost 40% of children had low iron stores (serum ferritin < 15 ng/mL); their ln-U-Cd was slightly higher ($0.11 [-0.06, 0.29]$) in comparison to children without ID. Possibly due to the modest sample size or the low U-Cd, the association with ID was not statistically significant. Menstruating girls and women are more at risk of developing ID. Several studies reported higher blood Cd among adolescent girls with lower serum ferritin values (Kim et al., 2017; Silver et al., 2013), although ID was not associated with urinary Cd in U.S. children (Silver et al., 2013).

Need for stricter standards for Cd.

Food is a recognized source of cadmium exposure and several health and environmental agencies have imposed standards to protect the public. Most recently, the European Food Safety Authority (EFSA) decreased its previous threshold of tolerable weekly intake (TWI) from food and water to $2.5 \mu\text{g/kg}$ body weight/week or $0.36 \mu\text{g/kg}$ body weight/day, which is based on chronic effects in adults (EFSA, 2009). More recent regulations establish even

lower TWI for Cd intake from foods for infants and young children (EU., 2014). The U.S. Environmental Protection Agency (EPA) sets the reference dose from food at 1.0 µg/kg/day and from water at 0.5 µg/kg/day (ATSDR, 1999). The MERCOSUR region (Argentina, Brazil, Paraguay and Uruguay), has established maximum limit of 0.2 mg/kg in imported and commercially available foods (Machado et al., 2015). Our study indicates that grain or grain product consumption may contribute to higher urinary Cd levels in children. Grains are a major source of energy and several countries have issued recommendations for the consumption of whole grains. In the U.S., daily 2-3 ounce-equivalents (32-48 g) for 4-8 year old children are recommended (USDA., 2015); in Scandinavian countries, the guidance is for 75 g/day/10 MJ of energy (Kyrø and Tjønneland, 2016). More monitoring and stricter standards for Cd in foods may be needed to limit exposure.

This study has several strengths. First, it not only examined the associations of urinary Cd concentrations with iron status, but also with specific nutrients and food groups, that are either rich in antagonists of Cd absorption or that serve as sources of Cd exposure, thus improving the translatability of our findings. As far as we are aware, with the exception of a study based on the U.S. NHANES (Riederer et al., 2013) and a recent study in Spain (Pérez et al., 2018), no other investigations of this sort exist in children. Second, dietary intake was averaged from two days of recall spaced at least 2 weeks apart (median 19 days), thus increasing the likelihood that the recalls represent a typical diet. Aids, probes, and having the children contribute to the recall, were deployed to minimize measurement error. Furthermore, 98% of children provided urine samples for Cd analysis; a robust response rate. Urinary Cd was measured using sensitive laboratory methods and ~80% of samples had detectable Cd levels.

On the other hand, the study had some limitations. First, due to the cross-sectional design, this study cannot establish a causal relationship between diets and urinary Cd. Second, we used urinary Cd, a biomarker of long-term (years) exposure, to represent body burdens of Cd. In contrast, our measure of dietary intakes were completed for most children within the space of one month. Blood Cd would have been a more suitable biomarker but we did not measure it in our study. Nevertheless, our findings are consistent with biological mechanisms. Third, water Cd concentrations did not predict urinary cadmium concentration in our study. Although this is consistent with reports from the U.S. general population (Riederer et al., 2013), we only measured Cd in household water and did not collect water from other sources, like schools. Also, our methods did not specifically include trace-element free collection cups. Fourth, our food groups were created, in part, based on children's consumption patterns. The creation of a single category encompassing many foods, necessitated by our data, precluded us from making conclusions about specific foods in relation to UCd. Finally, our findings cannot be generalized to groups with higher Cd exposure, e.g. populations consuming much rice or seafood.

5. CONCLUSIONS

In young children, iron and zinc intake may play some role in lowering the accumulation of Cd in children's bodies, even when Cd exposure is low. On the other hand, grains appear to

be an important source of Cd exposure in this setting. It would be important to conduct future studies among children with other dietary habits and nutritional status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We would like to thank nurses Delminda Ribeiro and Graciela Yuane for their help with sample collection and processing. We are also very grateful to the participating children and their families.

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. The authors declare no conflicts of interest.

7. REFERENCES

- Aguilera I, Daponte A, Gil F, Hernandez AF, Godoy P, Pla A, et al. Urinary levels of arsenic and heavy metals in children and adolescents living in the industrialised area of Ria of Huelva (SW Spain). *Environ Int* 2010; 36: 563–9. [PubMed: 20471088]
- Akesson A, Berglund M, Schutz A, Bjellerup P, Bremme K, Vahter M. Cadmium exposure in pregnancy and lactation in relation to iron status. *Am J Public Health* 2002; 92: 284–7. [PubMed: 11818307]
- Akkermans MD, van der Horst-Graat JM, Eussen SR, van Goudoever JB, Brus F. Iron and Vitamin D Deficiency in Healthy Young Children in Western Europe Despite Current Nutritional Recommendations. *J Pediatr Gastroenterol Nutr* 2016; 62: 635–42. [PubMed: 26488124]
- ATSDR. Toxicological Profile for Cadmium, Atlanta, GA, 1999.
- Barany E, Bergdahl IA, Bratteby LE, Lundh T, Samuelson G, Skerfving S, et al. Iron status influences trace element levels in human blood and serum. *Environ Res* 2005; 98: 215–23. [PubMed: 15820728]
- Berglund M, Akesson A, Nermell B, Vahter M. Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. *Environ Health Perspect* 1994; 102: 1058–66. [PubMed: 7713018]
- CDC. Fourth National Report on Human Exposure to Environmental Chemicals, Atlanta, GA, 2009.
- Ciesielski T, Weuve J, Bellinger DC, Schwartz J, Lanphear B, Wright RO. Cadmium exposure and neurodevelopmental outcomes in U.S. children. *Environ Health Perspect* 2012; 120: 758–63. [PubMed: 22289429]
- Donovan A, Roy CN, Andrews NC. The Ins and Outs of Iron Homeostasis. *Physiology* 2006; 21: 115–123. [PubMed: 16565477]
- EFSA EFSA. Cadmium in food: scientific opinion of the panel on contaminants in the food chain. *EFSA J* 2009; 980: 1–139.
- EFSA EFSA. Cadmium dietary exposure in the European population. *EFSA J* 2012; 10.
- Engstrom B, Nordberg GF. Factors influencing absorption and retention of oral ¹⁰⁹Cd in mice: age, pretreatment and subsequent treatment with non-radioactive cadmium. *Acta Pharmacol Toxicol* 1979; 45: 315–24.
- 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: 3127–32. [PubMed: 15514286]
- EU. EU. Commission Regulation (EU) No 488/2014 of 12 May 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in foodstuffs. In: Union E, editor. 488/2014, 2014.

- Exley K, Aerts D, Biot P, Casteleyn L, Kolossa-Gehring M, Schwedler G, et al. Pilot study testing a European human biomonitoring framework for biomarkers of chemical exposure in children and their mothers: experiences in the UK. *Environ Sci Pollut Res* 2015; 22: 15821–34.
- Fagerstedt S, Kippler M, Scheynius A, Gutzeit C, Mie A, Alm J, et al. Anthroposophic lifestyle influences the concentration of metals in placenta and cord blood. *Environ Res* 2015; 136: 88–96. [PubMed: 25460625]
- Fleming MD, Trenor CC, 3rd, Su MA, Foernzler D, Beier DR, Dietrich WF, et al. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat Genet* 1997; 16: 383–6. [PubMed: 9241278]
- Friedman LS, Lukyanova EM, Kundiev YI, Shkiryak-Nizhnyk ZA, Chislovska NV, Mucha A, et al. Anthropometric, environmental, and dietary predictors of elevated blood cadmium levels in Ukrainian children: Ukraine ELSPAC group. *Environ Res* 2006; 102: 83–9. [PubMed: 16729996]
- Fucic A, Plavec D, Casteleyn L, Aerts D, Biot P, Katsonouri A, et al. Gender differences in cadmium and cotinine levels in prepubertal children. *Env Res* 2015; 141: 125–31. [PubMed: 25529752]
- Gardner RM, Kippler M, Tofail F, Bottai M, Hamadani JD, Grandner M, et al. Environmental exposure to metals and children's growth to age 5 years: a prospective cohort study. *Am J Epidemiol* 2013; 177: 1356–67. [PubMed: 23676282]
- Gruenheid S, Cellier M, Vidal S, Gros P. Identification and characterization of a second mouse Nramp gene. *Genomics* 1995; 25: 514–25. [PubMed: 7789986]
- Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 1997; 388: 482–8. [PubMed: 9242408]
- Gustin K, Tofail F, Vahter M, Kippler M. Cadmium exposure and cognitive abilities and behavior at 10 years of age: a prospective cohort study. *Environ Int* 2018; 113: 259–68. [PubMed: 29459184]
- Jarup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Health effects of cadmium exposure-- a review of the literature and a risk estimate. *Scand J Work Environ Health* 1998; 24 Suppl 1: 1–51.
- Kim J, Shin M, Kim S, Seo J, Ma H, Yang YJ. Association of iron status and food intake with blood heavy metal concentrations in Korean adolescent girls and women: Based on the 2010–2011 Korea National Health and Nutrition Examination Survey. *J Nutr Health* 2017; 50: 350–360.
- Kippler M, Bottai M, Georgiou V, Koutra K, Chalkiadaki G, Kampouri M, et al. Impact of prenatal exposure to cadmium on cognitive development at preschool age and the importance of selenium and iodine. *Eur J Epidemiol* 2016; 31: 1123–1134. [PubMed: 27147065]
- Kippler M, Ekstrom EC, Lonnerdal B, Goessler W, Akesson A, El Arifeen S, et al. Influence of iron and zinc status on cadmium accumulation in Bangladeshi women. *Toxicol Appl Pharmacol* 2007; 222: 221–6. [PubMed: 17543360]
- Kippler M, Nermell B, Hamadani J, Tofail F, Moore S, Vahter M. Burden of cadmium in early childhood: longitudinal assessment of urinary cadmium in rural Bangladesh. *Toxicol Lett* 2010; 198: 20–5. [PubMed: 20466048]
- Kippler M, Wagatsuma Y, Rahman A, Nermell B, Persson LA, Raqib R, et al. Environmental exposure to arsenic and cadmium during pregnancy and fetal size: a longitudinal study in rural Bangladesh. *Reprod Toxicol* 2012; 24: 504–11.
- Kordas K, Burganowski R, Roy A, Peregalli F, Baccino V, Barcia E, et al. Nutritional status and diet as predictors of children's lead concentrations in blood and urine. *Environ Int* 2017; 111: 43–51. [PubMed: 29172090]
- 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 Total Environ* 2010; 408: 4488–94. [PubMed: 20619443]
- Kordas K, Queirolo EI, Manay 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]
- Krajcovicova-Kudladkova M, Ursinyova M, Masanova V, Bederova A, Valachovicova M. Cadmium blood concentrations in relation to nutrition. *Centr Eur J Publ Health* 2006; 14: 126–9.
- Kyro C, Tjonneland A. Whole grains and public health. *BMJ* 2016; 353: i3046. [PubMed: 27301398]

- Lin CM, Doyle P, Wang D, Hwang YH, Chen PC. Does prenatal cadmium exposure affect fetal and child growth? *Occup Environ Med* 2011; 68: 641–6. [PubMed: 21186202]
- Liu Y, Chen Q, Wei X, Chen L, Zhang X, Chen K, et al. Relationship between perinatal antioxidant vitamin and heavy metal levels and the growth and cognitive development of children at 5 years of age. *Asia Pac J Clin Nutr* 2015; 24: 650–8. [PubMed: 26693750]
- Machado I, Cesio MV, Dol I, Piston M. A Rapid Sample Preparation Method for the Determination of Cadmium and Lead in Spinach and Artichoke Leaves Using Ozone. *American Journal of Food Science and Technology* 2015; 3: 55–59.
- Madeddu R, Solinas G, Forte G, Bocca B, Asara Y, Tolu P, et al. Diet and nutrients are contributing factors that influence blood cadmium levels. *Nutr Res* 2011; 31: 691–7. [PubMed: 22024493]
- Manay N, Cousillas AZ, Alvarez C, Heller T. Lead contamination in Uruguay: the “La Teja” neighborhood case. *Rev Environ Contam Toxicol* 2008; 195: 93–115. [PubMed: 18418955]
- Mendil D, Unal OF, Tiizen M, Soylak M. Determination of trace metals in different fish species and sediments from the River Yeşilirmak in Tokat, Turkey. *Food Chem Toxicol* 2010; 48: 1383–92. [PubMed: 20226829]
- Molina-Villalba I, Lacasana M, Rodriguez-Barranco M, Hernandez AF, Gonzalez-Alzaga B, Aguilar-Garduno C, et al. Biomonitoring of arsenic, cadmium, lead, manganese and mercury in urine and hair of children living near mining and industrial areas. *Chemosphere* 2015; 124: 83–91. [PubMed: 25434277]
- Moynihan M, Peterson KE, Cantoral A, Song P, Jones A, Solano-Gonzalez M, et al. Dietary predictors of urinary cadmium among pregnant women and children. *Sci Tot Environ* 2017; 575: 1255–1262.
- NIH. Dietary Supplement Fact Sheet: Iron. 2017. NIH Office of Dietary Supplements, 2016.
- Olmedo P, Pla A, Hernandez AF, Barbier F, Ayouni L, Gil F. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. *Environ Int* 2013; 59: 63–72. [PubMed: 23792415]
- Poh BK, Ng BK, Siti Haslinda MD, Nik Shanita S, Wong JE, Budin SB, et al. Nutritional status and dietary intakes of children aged 6 months to 12 years: findings of the Nutrition Survey of Malaysian Children (SEANUTS Malaysia). *Br J Nutr* 2013; 110 (Suppl 3): S21–35. [PubMed: 24016764]
- Protano C, Astolfi ML, Canepari S, Vitali M. Urinary levels of trace elements among primary school-age children from Italy: the contribution of smoking habits of family members. *Sci Tot Environ* 2016; 557–8: 378–85.
- Perez R, Domenech E, Conchado A, Sanchez A, Coscolla C, Yusa V. Influence of diet in urinary levels of metals in a biomonitoring study of a child population of the Valencian region (Spain). *Sci Tot Environ* 2018; 618: 1647–57.
- Rango T, Jeuland M, Manthritilake H, McCornick P. Nephrotoxic contaminants in drinking water and urine, and chronic kidney disease in rural Sri Lanka. *Sci Tot Environ* 2015; 518–19: 574–85.
- Riederer AM, Belova A, George BJ, Anastas PT. Urinary cadmium in the 1999–2008 U.S. National Health and Nutrition Examination Survey (NHANES). *Environ Sci Technol* 2013; 47: 1137–47. [PubMed: 23253114]
- Roca M, Sanchez A, Perez R, Pardo O, Yusa V. Biomonitoring of 20 elements in urine of children. Levels and predictors of exposure. *Chemosphere* 2016; 144: 1698–1705. [PubMed: 26519801]
- Rojroongwasinkul N, Kijboonchoo K, Wimonpeerapattana W, Purttiponthanee S, Yamborisut U, Boonpradern A, et al. SEANUTS: the nutritional status and dietary intakes of 0.5–12- year-old Thai children. *Br J Nutr* 2013; 110 (Suppl 3): S36–44. [PubMed: 24016765]
- Ryu DY, Lee SJ, Park DW, Choi BS, Klaassen CD, Park JD. Dietary iron regulates intestinal cadmium absorption through iron transporters in rats. *Toxicol Lett* 2004; 152: 19–25. [PubMed: 15294343]
- Sandjaja S, Budiman B, Harahap H, Ernawati F, Soekatri M, Widodo Y, et al. Food consumption and nutritional and biochemical status of 0–5-12-year-old Indonesian children: the SEANUTS study. *Br J Nutr* 2013; 110 (Suppl 3): S11–20. [PubMed: 24016762]
- Satarug S, Vesey DA, Gobe GC. Current health risk assessment practice for dietary cadmium: data from different countries. *Food Chem Toxicol* 2017; 106 (Pt A): 430–45. [PubMed: 28602857]

- Shah F, Kazi TG, Afridi HI, Kazi N, Baig JA, Shah AQ, et al. Evaluation of status of trace and toxic metals in biological samples (scalp hair, blood, and urine) of normal and anemic children of two age groups. *Biol Trace Elem Res* 2011; 141: 131–49. [PubMed: 20526751]
- Shayeghi M, Latunde-Dada GO, Oakhill JS, Laftah AH, Takeuchi K, Halliday N, et al. Identification of an intestinal heme transporter. *Cell* 2005; 122: 789–801. [PubMed: 16143108]
- Shrier I, Platt RW. Reducing bias through directed acyclic graphs. *BMC Med Res Methodol* 2008; 8.
- Silver MK, Lozoff B, Meeker JD. Blood cadmium is elevated in iron deficient U.S. children: a cross-sectional study. *Environ Health* 2013; 12.
- Storelli MM, Normanno G, Barone G, Dambrosio A, Errico L, Garofalo R, et al. Toxic metals (Hg, Cd, and Pb) in fishery products imported into Italy: suitability for human consumption. *J Food Prot* 2012; 75: 189–94. [PubMed: 22221377]
- Suarez-Ortega MF, Mosquera M, Caicedo DM, De Plata CA, Mendez F. Nutrients intake as determinants of blood lead and cadmium levels in Colombian pregnant women. *Am J Hum Biol* 2013; 25: 344–50. [PubMed: 23559431]
- Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy Metals Toxicity and the Environment. *EXS* 2012; 101: 133–164. [PubMed: 22945569]
- Turgut S, Polat A, Inan M, Turgut G, Emmungil G, Bican M, et al. Interaction between anemia and blood levels of iron, zinc, copper, cadmium and lead in children. *Indian J Pediatr* 2007; 74: 827–30. [PubMed: 17901668]
- USDA. 2015–2020 Dietary Guidelines for Americans. U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015.
- Vacchi-Suzzi C, Kruse D, Harrington J, Levine K, Meliker JR. Is Urinary Cadmium a Biomarker of Long-term Exposure in Humans? A Review. *Curr Environ Health Rep* 2016; 3: 450–458. [PubMed: 27696280]
- WHO. Iron deficiency anaemia: assessment, prevention, and control United Nations Children's Fund, United Nations University, World Health Organization, Geneva, Switzerland, 2001.
- Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997; 65: 1220S–1228S; discussion 1229S–1231S. [PubMed: 9094926]
- Wittman R, Hu H. Cadmium exposure and nephropathy in a 28-year-old female metals worker. *Environ Health Perspect* 2002; 110: 1261–6. [PubMed: 12460807]
- Zailina H, Najibah H, Aiezzati AN, Praveena SM, Patimah I. Comparison of the health implications on the use of As and Cd contaminated water supply between urban and rural communities. *BioMed Res Int* 2014; 2014.
- Zhong MS, Jiang L, Han D, Xia TX, Yao JJ, Jia XY, et al. Cadmium exposure via diet and its implication on the derivation of health-based soil screening values in China. *J Expo Sci Environ Epidemiol* 2015; 25: 433–42. [PubMed: 25736161]

HIGHLIGHTS

- Cadmium exposure was low and did not differ between boys and girls.
- Higher consumption of grains was related to higher U-Cd.
- Micronutrient intake (iron but especially zinc) was related to lower U-Cd.
- Nutrients derived from common food sources cannot be studied individually vis-à-vis metal exposure.

Figure 1: Covariate-adjusted¹ associations between dietary intakes and ln-transformed U-Cd² concentrations in Uruguayan children.

¹Model adjusted for age, crowding in the home, maternal education (years), and household possessions; ²Concentration ($\mu\text{g/L}$) was adjusted for specific gravity of urine; natural-log-transformed for OLS modeling; ³Quantile of dietary intake, T=tertile, M=median; the lowest quantile serves as the reference group and is not shown. ⁴Foods rich in heme iron include red and white meats; ⁵Foods rich in non-heme iron include beans, peas, lentils, soy/bean burgers, spinach, broccoli, tomatoes, dried fruit; ⁶Grains include white and whole wheat flour, corn flour and starch, cereals, rice, granola bars and cereal bars, muesli, polenta, prepared dishes containing rice or grains; ⁷Root vegetables include carrots, beets, potatoes (including French fries), sweet potatoes, turnips; * $p < 0.10$; ** $p < 0.05$; *** $p < 0.01$.

Figure 2: Covariate-adjusted¹ associations between dietary intakes and the proportional odds of elevated U-Cd² among Uruguayan children.

¹Model adjusted for age, crowding in the home, maternal education (years), and household possessions; ²Concentration ($\mu\text{g/L}$) was adjusted for specific gravity of urine; ³Quantile of dietary intake, T=tertile, M=median, the lowest quantile serves as the reference group and is not shown; ⁴Foods rich in heme iron include red and white meats; ⁵Foods rich in non-heme iron include beans, peas, lentils, soy/bean burgers, spinach, broccoli, tomatoes, dried fruit; ⁶Grains include white and whole wheat flour, corn flour and starch, cereals, rice, granola bars and cereal bars, muesli, polenta, prepared dishes containing rice or grains; ⁷Root vegetables include carrots, beets, potatoes (including French fries), sweet potatoes, turnips; * $p < 0.10$; ** $p < 0.05$; *** $p < 0.01$.

Table 1.

Characteristics of the participating children, complete-case analysis.

Characteristic	N (%)	Mean $\pm$ SD Median [5%, 95%]	U-Cd ^I (μ g/L) Median [5%, 95%]
Age (mo)	279	80.1 $\pm$ 6.4	
71–78	35.8%		0.05 [0.01, 0.17]
79–83	31.2%		0.07 [0.01, 0.15]
84–105	33.0%		0.06 [0.03, 0.14]
Sex	279		
Male	44.4%		0.06 [0.02, 0.14]
Female	55.6%		0.06 [0.02, 0.17]
Either parent smokes	277		
No	47.3%		0.06 [0.02, 0.18]
Yes	52.7%		0.06 [0.02, 0.15]
Maternal education (y)	279	9.0 $\pm$ 2.7	
Some primary	18.6%		0.07 [0.02, 0.17]
Some secondary	57.0%		0.06 [0.01, 0.15]
College or higher	24.4%		0.06 [0.02, 0.17]
Maternal employment	275		
Employed	31.3%		0.06 [0.02, 0.17]
Unemployed	68.7%		0.06 [0.02, 0.15]
Household density ²	279	2.0 $\pm$ 0.7	
No crowding ³	74.4%		0.06 [0.02, 0.15]
Crowding	25.6%		0.06 [0.02, 0.17]
Household possessions	279	3.5 $\pm$ 1.1	
Median ⁴	43.4%		0.06 [0.02, 0.18]
< Median	56.6%		0.06 [0.01, 0.14]
Height (m)	276	1.22 $\pm$ 0.06	
BMI (kg/m ²)	276	16.9 $\pm$ 2.6	
Underweight ⁵	7.3%		0.06 [0.01, 0.17]
Normal	53.8%		0.06 [0.02, 0.15]
Overweight/obese	38.9%		0.06 [0.02, 0.17]
Energy intake (kcal/d)	279	2204 $\pm$ 564	
937 - 1919	33.3%		0.07 [0.02, 0.15]
1920-2420	33.3%		0.06 [0.01, 0.14]
2425 –4236	33.3%		0.06 [0.01, 0.16]
U-Cd ^I (μ g/L)	279	0.06 [0.02, 0.17]	---

^IAdjusted for specific gravity of urine²Household density=number of people sleeping in the house/number of bedrooms³Crowding= more than 2 persons per bedroom in the house

⁴Median possessions is 4

⁵Underweight=BMI-for-age Z-score <-1SD, Normal weight=BMI-for-age Z-score -1 to 1 SD, Overweight=BMI-for-age Z-score >1SD.

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Table 2.
Association between water and urinary Cd concentrations among Uruguayan children (n=191).

U-Cd ¹ Median [5%, 95%]	Ln-U-Cd ¹ β [95% CI]		Proportional odds of elevated of U-Cd ² OR [95% CI]	
	Unadjusted	Covariate-adjusted ³	Unadjusted	Covariate-adjusted ³
Water cadmium ($\mu\text{g/L}$)				
0.001-0.01	0.01 [0.003, 0.02]	Ref.	Ref.	Ref.
0.01-0.04	0.04 [0.03, 0.06]	-0.11 [-0.36, 0.13]	0.53 [0.27, 1.02]*	0.58 [0.30, 1.14]
0.05-9.8	0.09 [0.06, 1.8]	-0.01 [-0.23, 0.21]	1.15 [0.62, 2.13]	1.22 [0.65, 2.29]

¹ Urinary Cd concentration ($\mu\text{g/L}$) adjusted for specific gravity of urine

² Proportional odds determined in ordinal regression modeling U-Cd as a 4-category outcome variable

³ Model adjusted for age, crowding in the home, maternal education (years), and household possessions

* p<0.1.