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Association of blood lead levels with urinary F_2-8α Isoprostane and 8-hydroxy-2-deoxy-Guanosine concentrations in first-grade Uruguayan children

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Abstract

Oxidative stress (OS) is a potential molecular mechanism for lead-induced toxicities, yet, we have limited understanding of the relation between low-level lead (Pb) exposure and OS, especially in children. This cross-sectional study examines the association between blood lead level (BLL) and two OS markers—urinary F_2-8α isoprostane or isoprostane (a marker of lipid peroxidation) and 8-hydroxy-2-deoxy-Guanosine or 8-OH-dG (a marker of DNA damage) in 211 children, aged 5–8 years, from Montevideo, Uruguay. The role of dietary intakes of vitamin C and zinc in modifying the relation between BLL and OS was also examined. The mean (SD) BLL of the study children was 4.7 (2.2) μg/dL, with 30.2% children having BLL ≥5 μg/dL, the current reference level set by the US Centre for Disease Control for identifying, monitoring and management of children with elevated BLL. In covariate-adjusted analysis, there was a weak positive association between BLL and urinary isoprostane (adjusted for specific gravity) [$\beta = 0.09$, $p < 0.1$]. No association was found between children's BLL and urinary 8-OH-dG. Interactions between dietary intakes of vitamin C or zinc and BLL on OS biomarkers were not consistent. However, when BLL and vitamin C or BLL and zinc were modeled together, BLL was independently associated with isoprostane concentration [$\beta = 0.10$, $p < 0.05$] but vitamin C or zinc intake was not. These findings suggest that there may be a potential adverse effect of BLL on OS in children with low-level Pb exposure. There is a need to study the effects of Pb on other OS measures, as well as the role of OS in mediating low-level Pb toxicity on functional outcomes.

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Keywords

lead; Pb; isoprostane; 8-hydroxy-2-deoxy-Guanosine; oxidative stress; child; school-age; lipid peroxidation; DNA damage; Uruguay

1. INTRODUCTION

Lead (Pb) exposure poses a threat to children's physical and cognitive growth and development during and beyond childhood (Bellinger et al., 2011, Mazumdar et al., 2011). Although there may be several mechanisms by which Pb exerts its toxic effects, oxidative stress (OS) has been suggested as one molecular mechanism for Pb-induced toxicities (Ahamed & Siddiqui, 2007). Lead has the ability to induce oxidative imbalance by multiple mechanisms, including generating ROS, affecting the antioxidant defense system, and altering the structure and functions of cellular membranes (Ahamed & Siddiqui, 2007).

Evidence from experiments in animals and observational studies in occupationally-exposed adults suggest that exposure to high levels of Pb can induce the generation of lipid peroxidation products, alter the activities of antioxidant enzymes and cause changes to nucleic acid bases or structural damage to DNA molecules (Berrahal et al., 2011; Haleagrahara et al., 2011; Sandhir et al., 1995; Bizon et al., 2013; Garcon et al., 2007; Grover et al., 2010; Kasperczyk et al., 2012; Khan et al., 2008; Malekirad et al., 2010; Olewińska et al., 2010; Permpongpaiboon et al., 2011; Bizon et al., 2013; Garcon et al., 2004; Garcia-Leston et al., 2012; Khan et al., 2008; Moro et al., 2010; Permpongpaiboon et al., 2011). Further, among non-occupationally exposed non-pregnant US adults with low blood lead level (BLL), a positive association was reported between BLL and serum γ-glutamyltranferase (GGT), a liver enzyme and an early marker of OS, after adjusting for age, sex, race and socio-economic status (Lee et al., 2006). In contrast, the relationship between BLL and OS in children is not well studied. Few studies that have reported higher levels of OS in children with high levels of exposure to Pb (Ahamed et al., 2008; Cabral et al., 2012, Jin et al., 2006), did not control for factors such as exposure to other metals, age, body-weight, socio-economic or nutritional status, all of which may potentially affect oxidative status.

Given the paucity of research among children with respect to low-level Pb exposure and OS, our aim was to examine the association between BLL and two measures of OS: urinary F_2-8α isoprostane (isoprostane) and 8-hydroxy- 2 deoxy Guanosine (8-OH-dG) in first-grade children living in Montevideo, Uruguay. Isoprostanes are generated from the oxidation of tissue phospholipids (mostly arachidonic acid) and increased concentrations have been linked to chronic diseases (Basu, 2008; Kanaya et al., 2011; Kaviarasan et al., 2009). In particular, chronic conditions such as cardiovascular disease, elevated blood pressure, and hypertension, associated with elevated levels of isoprostane, have also been related to lead exposure (Bushnik et al., 2014; Martin et al., 2006; Navas-Acien et al., 2007). On the other hand, 8-OH-dG is produced by the hydroxylation of the guanosine moiety of a nucleic acid base, and is an established marker of DNA oxidation (Valavanidis et al., 2009). The production of both isoprostane (Holt et al., 2009; Tomey et al., 2007) and 8-OH-dG (Hong et al., 2013; Sram et al., 2012; Engstrom et al., 2010) may be affected by nutrients that

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modulate the biological pathways for the generation of free radicals or influence the balance between free radicals and antioxidant capacity in the body. We investigated the role of two antioxidants, vitamin C and zinc, for which dietary intake information was available, on the association between BLL and urinary concentrations of isoprostane, and 8-OH-dG in first-grade children. We tested the hypothesis that higher intake of vitamin C or zinc would attenuate the effects of BLL on OS measures.

2. METHODS

2.1. Participant recruitment

All first grade children attending private elementary schools located in different neighborhoods of Montevideo were eligible for the study. Montevideo is a unique setting for the study because children there have been exposed to low-levels of environmental Pb (Queirolo et al., 2010). The advertisement for the study was made via posters, radio, television, and newspaper announcements. In addition, all private elementary schools in the selected neighborhoods where Pb exposure was suspected or documented were identified and school principals were contacted to briefly explain the study and invite the school's participation. The schools in Montevideo are divided according to socio-economic status (SES) of the students/families they serve. For the purpose of this study, schools from low-to-middle SES were contacted. In total, nine schools agreed to participate in the study. Posters advertising the study were hung up at the school, and an informational meeting for the parents was scheduled. At the meetings, general information on childhood Pb exposure was provided, and the rationale and procedures of the study were explained. Parents had the option of providing consent at the meeting or returning the signed forms after consulting with other family members.

The sole exclusion criterion was a previous diagnosis of Pb poisoning (BLL >45 μg/dL). None of the children were excluded on this basis. Of the 410 eligible children, 211 finally enrolled into the study. The study was approved by the Ethics Committee for Research Involving Human Participants at the Pennsylvania State University (IRB# 28597), the Catholic University of Uruguay (IRB# B041108), and the University of the Republic of Uruguay (IRB approval date: March 12, 2009).

2.2. Socio-demographic information

Parents/caregivers who agreed to participate in the study were invited for a meeting at the school to fill out a questionnaire about socio-demographic characteristics of the family, the child's medical history and the home environment. Specifically, caregivers were asked to provide detailed information about their age, education, occupation, smoking history, and family structure. To assess socio-economic condition and home environment of the families, caregivers were asked to fill out questions on their monthly income, daily expenditures on food and clothes, home ownership, number of rooms, number of persons living in the house, including number of children < 5 years of age; and family possession of 12 household items like TV, video player, DVD player, computer, video games, radio, sound equipment, refrigerator, washer, home phone, cellular phone and car. The questionnaires were self-administered but the research staff provided assistance if needed.

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2.2. Blood and urine collection

Fasting blood was collected by a phlebotomy nurse at the school during a morning visit. Children brought in their first void urine samples on the morning of the clinic visit in cups that had been provided to them on a prior occasion. These had been rinsed repeatedly with 10% HNO_3 and deionized water, and then air dried. The urine samples were transported on ice to the Faculty of Chemistry at the University of the Republic on the day of collection. There, urine aliquots for measuring isoprostane were made immediately and stored at −80°C in the presence of 0.005% BHT (Beta Hydroxy Toluene) to prevent oxidative formation of isoprostane. The rest of the aliquots were stored at −20°C in 10 mL plastic tubes previously rinsed with 10% HNO_3 and deionized water. All urine samples were shipped on dry ice and stored at −80°C (for isoprostane) and −20°C (for 8-OH-dG) until analysis at the Department of Nutritional Sciences, the Pennsylvania State University (USA). Specific gravity of each urine sample was measured using a portable specific gravity refractometer (PAL 10S, Atago Inc, USA) on the day of the collection. Each day, the refractometer was calibrated by pipetting 2–3 drops of tap water onto the prism surface and taking a reading.

2.3. Biochemical analysis

Blood Pb concentrations were measured at the CEQUIMTOX (Specialized Center for Chemical Toxicology), at the Faculty of Chemistry of the University of the Republic 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 and 0.8 μg/dL for graphite furnace AAS techniques, respectively. Analytical conditions were validated with standard quality assurance/quality control (QA/QC) procedures (Parson and Chisolm, 1997). The laboratory participates in CDC's Lead and Multi-Element Proficiency Program and the Interlaboratory Program of Quality Control for Lead in Blood, Spain.

Urinary isoprostane was analyzed at the Pennsylvania State University using a commercially available competitive enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI). Prior to this analysis, all samples were purified using a solid phase extraction (SPE) method, to remove any organic solvents that could interfere with the proper measurements of isoprostane, as suggested by the manufacturer. Urine samples were thawed at room temperature and vortexed vigorously. Then, one ml of the sample was diluted with 2.5 ml of 1M acetate buffer. For each sample, a 6 ml column (C-18 SPE cartridge, Cayman Chemical, Ann Arbor, MI) was activated by rinsing with 5 ml HPLC-grade methanol and 5 ml of double distilled water. The columns were not allowed to dry. Next, the diluted samples were passed through the activated columns, followed by a rinse with 5 ml of double distilled water and 5 ml HPLC-grade hexane. Isoprostane in each sample was eluted with 5 ml ethyl acetate containing 1% methanol. The eluted isoprostane in ethyl acetate solution was stored in a glass vial at −80°C until analysis. On the day of the analysis, samples were brought to room temperature and the ethyl acetate fraction was evaporated under a stream of dry nitrogen. The dried samples were then reconstituted with a buffer provided in the EIA kit. The assay was performed with the reconstituted samples following the manufacturer's instructions. Standards and samples were run in duplicate with an average coefficient of

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variance (CV) of 5.4%. Finally, isoprostane levels were adjusted to the mean specific gravity (1.023) to correct for variation in fluid intake.

For the analysis of 8-OH-dG, aliquots of 1 ml of urine were brought to room temperature and thawed urine specimens were centrifuged at 4000 × g for 10 min to remove any sediment. Urinary 8-OH-dG was analyzed by a commercially available competitive enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI). Samples were diluted 100 fold with an EIA buffer provided by the manufacturer. Standards and samples were run in duplicate with a CV of 7%.

As indicated by the manufacturer, interference by other urinary components in measuring 8-OH-dG by the assay is infrequent for urine samples. Sample purification has only been recommended in case of a 20% or greater disparity between the concentrations of 8-OH-dG in two different dilutions of the same sample. To test for interference, ten random test samples were diluted to two different levels (varied between samples to fit the data within acceptable range) and the final concentrations of 8-OH-dG were compared. On average, the concentrations differed by 10% in two different dilutions of the same sample and therefore no purification was done. Specific gravity adjustment was also done for urinary 8-OH-dG levels.

Serum ferritin concentrations were determined in duplicate by an immunoradiometric assay kit (Coat-A-Count Ferritin IRMA; SIEMENS Diagnostic Products, USA). Intra-assay and inter-assay CVs were 4.2% and 9.5% respectively. Concentration of C-Reactive Protein (CRP) was measured to identify the presence of subclinical inflammation/infection in the study children. CRP was analyzed in duplicate using an ELISA technique (Erhardt et al., 2004) with an intra-assay and inter-assay CV of 4.9% and 8%, respectively. Serum ferritin concentrations were adjusted for the presence of inflammation by adjusting for serum CRP concentration (Thurnham et al., 2010).

2.3. Anthropometric measurements

Children were 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 software (WHO, 2010). Overweight (BMI-z-score> +1 SD) and obesity (BMI-z-score> +2 SD) were defined according to WHO standards (deOnis et al., 2007).

2.4. Dietary assessment

To determine children's dietary intakes, two 24- hour dietary recalls were conducted by five 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, particularly with the recollection of food consumed at school. 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

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weeks later, and fell either on a weekday or a weekend. Three separate contact attempts were made by telephone to complete the phone interview. The recall was not conducted if all attempts went unanswered or the primary caregiver was not available.

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 measurement cups were used during the interview with the mothers or caregivers to facilitate food portion recalls, and to quantify the amount and volume of foods/beverages consumed (Compendio de Referencias Prácticas, Oficina del Libro FEFMUR, Montevideo, 2002; Vázquez and Witriw, 1997). 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?” “Did he/she have anything before going to bed?” were asked to get accurate dietary information. All the 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 (altogether 342 unique items). The 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. Milk distributed in school-based lunch programs is also fortified with iron under the same law. Calculations of nutrient intakes for each participant were derived from the database. Finally, the nutrient values obtained from the database were adjusted for energy intake by the residual method (Willet et al., 1997).

2.5. Statistical Analysis

All statistical analyses were performed using STATA 12.0 (StataCorp, College Station, TX, USA).

Complete case analysis—A series of univariate and multivariate regression models was built to test the independent and interactive effects of BLL, and intakes of vitamin C and zinc on urinary measures of OS (isoprostane and 8-OH-dG). First, to test whether Pb exposure was independently associated with OS, BLL was entered as a continuous independent variable in unadjusted and adjusted regression analyses, conducted separately for isoprostane and 8-OH-dG. Subsequently, energy-adjusted intakes of vitamin C and zinc (both as tertiles) were entered as independent variables (without BLL) in regression analyses to test the relation between these nutrients and OS. For adjusted analyses, all models included a set of covariates. Covariates were chosen based on the previous literature or if they were associated with isoprostane, 8-OH-dG or BLL in bivariate regression analyses ($p < 0.15$). Covariates for the multivariate models included family possession of household items (dichotomized at the median for the number of items possessed out of 12 items: ≤ 8 vs >8 items), crowding at home (number of people/room), parental involvement in jobs with potential for metal exposure (not-involved vs involved), mother’s current smoking status (non-smoker vs smoker), child’s BMI [weight (kg)/height (m^2)] and CRP-adjusted ferritin concentration (ng/mL). To assess the potential occupational exposure to metals, parents

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were asked to indicate whether they performed jobs that involve chemicals or metals, recycling, auto-repair, plumbing and paint work for 6 months or more. The responses were coded as: “0” if neither of the parents was involved, “1” if one of the parents was involved and “2” if both parents were involved. The total score was derived by adding the responses to all the items and ranged from 0 to 8. Because a large proportion of the parents (66.9%) reported no involvement in jobs listed in the questionnaire, total “occupational exposure” score was collapsed into not-involved vs involved. All the socio-demographic covariates were selected and created from the self-reported parental questionnaire.

The potential interactions between BLL and intakes of vitamin C, and zinc were tested in separate regression models for isoprostane and 8-OH-dG. To test the interactive effects of BLL and vitamin C, two independent variables (BLL and vitamin C) plus the interaction term (BLL x vitamin C) were included in a model. Similarly, BLL, zinc, and the interaction between BLL and zinc were modeled together. Interaction terms were created by crossing the individual nutrient tertiles with BLL as a continuous variable (centered at mean value). Non-significant interactions ($p > 0.15$) were removed and regression models were re-run testing BLL and vitamin C, and BLL and zinc intake together.

Missing data analysis—In the complete-case analysis, the outcome variables and the predictors were subject to missing data. To avoid potential bias and loss of power from complete-case analysis, muiltivariate multiple imputation was used to impute missing variables. The imputation model included the primary outcome variables (isoprostane and 8-OH-dG), BLLs, other biomarkers such as hemoglobin (measured by portable hemoglobinometer, Hemocue) and serum ferritin concentrations, and all the socio-demographic variables from all 211 children who completed the study. Imputation by chained equations (command “mi ice”) of STATA version 12.0 (StataCorp, College Station, TX) was conducted (Royston and White, 2011) in 10 cycles. We created 50 imputed datasets for our analyses. Regression models were rerun using the imputed data set.

3. RESULTS

3.1. Characteristics of study children

The socio-demographic, biological and dietary data of the study children are presented in Table 1. The mean (SD) age of the children was 6.8 (0.6) years. Urine samples for analyses of isoprostane and 8-OH-dG were available for 186 and 188 children, respectively. In total, 182 children provided blood for Pb analysis and information on daily intake of nutrients was available for 187 children. The study children had a mean (SD) BLL of 4.7 (2.2) μg/dL, with 30.2% children having elevated BLL (≥ 5 μg/dL). The mean (SD) urinary isoprostane and 8-OH-dG concentrations, adjusted to specific gravity, were 1.5 (1.2) ng/ml and 48.8 (33.1) ng/ml, respectively. All children had urinary concentrations of isoprostane and 8-OH-dG above the limits of detection (2.7 pg/mL & 30 pg/mL, respectively). Only 3% of the study participants had anemia (Hb< 11.5 g/dL), whereas more than half of the children (61.2%) had iron-deficiency (CRP-adjusted serum ferritin <15 ng/ml). The mean (SD) BMI was 16.9 kg/m^2, with 19.8 % of the children being overweight (BMI-z-score> +1 SD) and another 19.8% being obese (BMI-z-score> +2 SD) according to WHO reference values (deOnis et al., 2007).

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More than half of the mothers (65.7%) reported having some secondary education. Majority (66.9%) of the parents indicated that they were not engaged in jobs with potential for exposure to Pb, such as jobs that involve chemicals or metals, recycling, auto-repair, plumbing and paint work for six months or more. However, more than half of the fathers (51.8%) reported employment in jobs that could be a source of exposure to metals in general (construction, factory work, print shop, mechanic and driver). The average number of luxury items in the households of the study children were 8.3 out of 12 items. About 36.4% of the families did not own a house and 22.6% of the families had more than two people living in one room.

Mean (SD) calorie intake, averaged over two-24 hour recalls, was 2279 (602) Kcal. Mean (SD) energy-adjusted vitamin C and zinc intakes were 58 (53) mg/day and 5 (2) mg/day, respectively. While 34.2% children did not meet the Recommended Dietary Allowance (RDA) (Institute of medicine, USA) for zinc intake of 4.1 mg/day, approximately 19.3% of study children did not meet the RDA of 25 mg/day for vitamin C.

3.2. Association between blood lead levels, oxidative stress measures and nutrient intake (vitamin C and zinc)

When the association between BLL and OS was assessed in an unadjusted regression analysis, there was a weak positive association between BLL, and isoprostane (Table 2, Model 1). No association was observed between BLL and 8-OH-dG. The intakes of vitamin C and zinc were not associated with isoprostane (Table 2, Model 2). On the other hand, in the model with 8-OH-dG as an outcome, higher zinc but not vitamin C intake was associated with lower levels of 8-OH-dG (Model 2). There were no consistent BLL-by-nutrient intake interactions (data not shown). In the unadjusted main effects models, BLL but not vitamin C or zinc intake was significantly associated with isoprostane (Table 2, Models 3 & 4). In contrast, there was an inverse relation between zinc intake and 8-OH-dG concentration, but no significant association of BLL with 8-OH-dG in the unadjusted main effects model (Model 4).

Multivariate regression analyses with OS measures as outcome variables showed that each 1 μg/dL of BLL was associated with a 0.09 ng/ml higher isoprostane concentration ($p < 0.1$) (Table 2, Model 1). No significant association was observed between BLL and 8-OH-dG concentrations.

When the intakes of vitamin C and zinc were tested in models adjusted for covariates to test the independent association between the two nutrients and OS, there was a weak inverse association between zinc intake and 8-OH-dG (Table 2, Model 2). Specifically, compared to the lowest tertile of zinc intake, children in the highest tertile had about 13 ng/ml lower 8-OH-dG concentration ($p < 0.1$).

As with unadjusted associations, there were no consistent BLL-by-nutrient interactions on oxidative stress markers, thus all analyses were repeated testing the main effects of BLL and vitamin C, (Table 2, Model 3) and BLL and zinc (Table 2, Model 4). Each 1 μg/dL increase in BLL was associated with 0.10 ng/ml [95% CI: 0.01, 0.19] and 0.09 ng/ml [95% CI: 0.01. 0.18] increase in isoprostane concentration ($p < 0.05$) when modelled together with vitamin

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C and zinc, respectively (Models 3 & 4). Intake of vitamin C or zinc was not associated with isoprostane. While there was no association between BLL and 8-OH-dG, children in the highest tertile of zinc had about 14 ng/mL lower 8-OH-dG concentration than children lowest tertile of zinc intake [95% CI: −23.31, −0.67] ($p < 0.05$) (Model 4).

3.3. Missing data analysis

When we repeated the regression models with the imputed dataset, we found similar results as with the complete-case analyses (Table 3).

4. DISCUSSION

In a study of first-grade children from Montevideo, Uruguay, we examined the relation between children's BLLs and OS measured as urinary concentrations of isoprostane (lipid peroxidation product) and 8-OH-dG (DNA damage marker), and explored the potential interactions of antioxidants such as vitamin C or zinc and BLL on children's OS. We found a weak positive association between children's BLL and urinary isoprostane after adjusting for socio-demographic and biological factors. In contrast, there was no statistically significant association between BLL and concentration of urinary 8-OH-dG. We did not find consistent interactions between BLL and intakes of vitamin C or zinc.

Ours is one of the few studies in children to test whether low-level Pb exposure is associated with OS and whether nutritional factors attenuate this association. A recent study by Martinez and colleagues (2013), also tested the association between low-level Pb exposure and OS measured as the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in 0 – 14 year old Argentinian children with a mean (SD) BLL of 2.6 (0.3) μg/dL. They did not find any statistically significant relation between children's BLL and CAT or SOD activities. However, they made no adjustments for socio-demographic, biological or nutritional factors, which may influence their findings. Previously, there were few reports of a positive relation between BLL and OS, measured as lipid peroxidation in children (Cabral et al., 2012; Ahamed et al., 2011, 2008, 2006, and 2005). For example, Cabral and colleagues (2012) reported a positive correlation between BLL and plasma malondialdehyde (MDA) concentration (marker of lipid peroxidation) in 1–16 year old children living near a landfill. However, the authors did not perform any adjusted analysis to take into account the other possible predictors of OS or BLL. Moreover, in the case-control study by Cabral and colleagues, children's BLL ranged from 3.25 μg/dL to 37.34 μg/dL, which is higher than BLL observed in most general pediatric populations including our study (BLL range: 0.8 – 13.2 μg/dL). In contrast, although a cross-sectional study in 3 to 6 year old preschool children with a mean (SD) BLL of 6.98 (1.75) μg/dL found a significantly higher plasma MDA concentrations in children with BLL≥ 10 μg/dL than children with BLL< 10 μg/dL (Jin et al., 2006), there was no dose-response relationship. The authors attributed the null finding to the induction of low to moderate levels of free radicals at a low BLL (< 10 μg/dL), which may be compensated by the antioxidant defense system, thus attenuating the extent of oxidative damage. Notably, in their statistical analysis, Jin and colleagues did not report of any adjustment for covariates such as intake/status of antioxidants.

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On the other hand, a positive association between Pb exposure and lipid peroxidation has been observed in studies with occupationally exposed adults (Bizon et al., 2013; Garcon et al., 2007; Garcia-Leston et al., 2012; Khan et al., 2008; Moro et al., 2010; Permpongpaiboon et al., 2011) and in few reports in the general adult population (Ahamed et al., 2009; Serafim et al., 2012). Most of the studies in occupationally exposed adults that assessed lipid peroxidation reported significant positive correlation between BLL and plasma MDA (e.g. Bizon et al., 2013; Garcon et al., 2007; Kasperczyk et al., 2012; Khan et al., 2008; Moro et al., 2010; Permpongpaiboon et al., 2011), while one also reported a positive correlation between BLL and total peroxides and conjugated diene (Permpongpaiboon et al., 2011). Among very few studies in non-occupationally exposed adults, Serafim and colleagues (2012) observed that Pb levels in the placenta were positively correlated with lipid peroxidation (LPO)- expressed as a sum of MDA and 4-hydroxyalkenals (4-HNE) per gram of total protein in 17–40 year old healthy Portuguese pregnant women. Similarly, placental Pb were positively related to placental MDA concentrations in Indian pregnant women (Ahamed et al., 2009). On the other hand, Pollack and colleagues (2012) did not observe any relation between BLL and lipid peroxidation markers such as plasma F_2-8α isoprostane, 9-hydroxy-10,12-octadecadienoic acid (9-HODE), 13-hydroxy-9,11- octadecadienoic acid (13-HODE) and TBARS in 18–44 years healthy non-pregnant women with a median (range) BLL of 0.86 (0.67–1.20) μg/dL after adjusting for relevant covariates such as age, BMI, smoking status and race. That study did include measures of income, education, physical activity, parity, dietary iron, shell sh, vegetables, dietary selenium, calcium, and total energy intake as potential confounders but these did not alter the effect estimates.

Our finding of a null association between BLL and 8-OH-dG, a DNA oxidation marker, is consistent with the available literature among adults. In occupationally exposed workers from a glass factory, Lin and colleagues (2012) did not find any significant association between urinary Pb levels and 8-OH-dG after adjusting for age, smoking status and alcohol consumption. Similarly, BLL was not associated with urinary 8-OH-dG in Bangladeshi pregnant women (Engstrom et al., 2010). To our knowledge, no studies in children examined the association between BLL and 8-OH-dG to compare with our findings. However, four studies in children examined the relation between BLL and DNA damage or DNA repair as measured by the comet assay (also known as single cell gel electrophoresis) or other methods (such as micronuclei (MN) and sister chromatid exchange (SCE) levels in peripheral lymphocytes) (Jasso-Piñeda et al., 2012, Mendez et al., 2008, Mielzynska et al., 2006; Yanez et al., 2003) and found higher levels of DNA damage in children with higher BLL compared to children with lower BLL. In addition, Mielzynska and colleagues (2006) observed a direct association between BLL and DNA damage as measured by number of micronuclei per 1000 cells in peripheral lymphocytes in 5–14 year old Polish children with a mean BLL of 7.7 μg/dL. The authors adjusted the analysis for children's age, sex, parental education, exposure to environmental tobacco smoke (ETS), types of household heating system as a proxy indicator of indoor emission from coal-burning stoves and biomarkers of polycyclic aromatic hydrocarbon (PAH) exposure (urinary levels of 1-hydroxypyrene and PAH-DNA adducts).

While comet assay or other methods used by other studies may be more direct measures of DNA damage, 8-OH-dG is an established marker of oxidative DNA damage and has been

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used in many studies of children (Dziaman et al., 2007; Svecova et al., 2009, Szaflarska-Poplawska et al., 2010). In this study, we did not monitor the extent of air pollution or ETS, which have been found to affect 8-OH-dG concentrations (Svecova et al., 2009). However, we tested the relation between self-reported smoking status of the parents, a crude measure of ETS, and children's urinary 8-OH-dG levels. Having parents who smoked was associated with higher levels of 8-OH-dG than having parents who did not smoke, but this association failed to reach statistical significance. It is possible that low-level Pb exposure, such as observed in our study, does not induce significant production of 8-OH-dG or that there may be other unmeasured factors, such as exposure to other toxicants which could better explain the variation in 8-OH-dG concentrations of this group of children.

Although, we did not find significant associations between the intake of vitamin C and isoprostane or 8-OH-dG, there was an inverse relation between zinc intake and 8-OH-dG. In the covariate-adjusted model children in the highest tertile of zinc intake had about 13 ng/ml less 8-OH-dG than children in the lowest tertile of zinc intake. Children who did not meet RDA for zinc intake for the age group (4 mg/day) also had higher 8-OH-dG than children who met the RDA (data not shown). Zinc has antioxidant properties and provides defense against OS indirectly (Bray & Betiger, 1990, Powell, 2000). Marginal dietary zinc deficiency has been shown to increase OS, impair DNA integrity, and increase DNA damage in rats (Nair et al., 2005; Song et al., 2009; Yousef et al., 2002). Notably, majority of the children in our study met the recommended dietary allowance (RDA) for the age group except for zinc (34.2% did not meet the RDA) and to some extent vitamin C (19.3% did not meet RDA).

Our findings need to be considered in light of some limitations. First, many variables, particularly socio-demographic variables, were subject to missing data due to non-response. Therefore the sample size was comparatively small in the final unimputed multivariate models. Our inconsistent findings on interaction between BLL and nutrient may also be due to lack of power. However, we repeated the analyses in multiple imputed datasets to handle the problem of missing data and our results were consistent with complete-case estimates. Nonetheless, our findings need to be tested in a larger sample in future studies. Second, we did not have information on children's exercise routine or physical activity levels, and could not control for those factors in the analysis. Physical activity may influence oxidative status in the body (Llorente-Cantarero et al., 2012; Tomey et al., 2007) and both inadequate physical activity (Llorente-Cantarero et al., 2012) and acute exercise (Jenkins, 2000) may increase OS. However, we did adjust for other physical measures such as BMI. Third, we used ELISA methods for analyses of isoprostane and 8-OH-dG concentrations in urine. Chromatographic methods (HPLC or GC-mass spectrometry) are considered a gold standard for measuring 8-OH-dG, but a consistently high correlation has been observed among the urinary 8-OH-dG values analyzed by chromatographic methods and ELISA (Yoshida et al., 2002). Finally, we collected nutrient intake data, but except for iron, we did not have data on biomarkers for those nutrients. As with other diet assessment methods, dietary recalls are subject to memory and respondent bias. To reduce this, we facilitated recall by probing about snacks and specific meals.

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Our study has several strengths. This is one of the few studies in children to test the relation between BLL and OS at a level of Pb exposure normally observed in the general population. This is important because the relevance of Pb-induced OS in general pediatric population with low environmental exposure to Pb is not clear. Most of the studies have typically been conducted at higher doses than the concentrations observed in the general population. Next, unlike many previous studies, we made extensive covariate adjustments in our regression models to better explain the variability in children's OS measures. Third, we evaluated the potential interactions between two antioxidants and BLL on OS. These interactions have not been tested in previous studies.

5. CONCLUSION

In 5–8 year old Uruguayan children, we found some evidence of an association between BLL and urinary isoprostane, but not urinary 8-OH-dG. Dietary intakes of vitamin C and zinc did not modify the relation between BLL and OS. More studies are required particularly in children with low-level environmental Pb exposure to confirm our results. In future, research should focus on more detailed investigation of OS measures including the status of antioxidant enzymes, non-enzymatic antioxidants, along with lipid peroxidation, DNA and protein damage products. This will give a comprehensive understanding of the nature and extent of damage. In addition, studies should examine whether OS is a potential mediator in the relation between Pb exposure and adverse biochemical consequences such as inflammation or functional outcomes such as neurodevelopmental problems, hypertension and diabetes. Furthermore, the role of dietary intake of antioxidant nutrients such as vitamin A, E, C, selenium, and zinc along with specific food groups in protecting against Pb-induced OS should be investigated.

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Highlights

- Oxidative stress (OS) is one potential mechanism for lead toxicity.
- Association between blood Pb level (BLL), isoprostane, and 8-hydroxy-2-deoxy Guanosine (8-OH-dG) was evaluated.
- A weak positive association was observed between BLL and isoprostane, a marker of lipid peroxidation.
- No significant relationship was found between BLL and 8-OH-dG, a marker of DNA damage.

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Table 1

Characteristics of study participants

Variables	n	All Subjects Mean ± SD or %	Blood lead concentration, µg/dL: *Tertile 1* 0.8 – 3.2 (n= 62) Mean ± SD or %	*Tertile 2* 3.3 – 4.9 (n= 65) Mean ± SD or %	*Tertile 3* 5.0 – 13.2 (n= 55) Mean ± SD or %
Biological factors					
Age (y)	204	6.8 ± 0.6	6.7 ± 0.5	6.9 ± 0.6	6.8 ± 0.6
Sex (female)	211	43.1%	33.8%	36.4%	30.0%
Blood lead concentration (µg/dL)	182	4.7 ± 2.2	2.6 ± 0.6	4.6 ± 0.5	7.2 ± 1.9
≥ 5 µg/dL		30.2%	---	---	---
Hemoglobin (g/dL)	184	13.4 ± 1.1	13.4 ± 1.1	13.3 ± 1.2	13.4 ± 1.0
<11.5 g/dL		3.0%	20.0%	40.0%	40.0%
Serum ferritin (ng/ml)[1]	174	14.3 ± 13.2	14.4 ± 14.8	14.8 ± 14.3	12.9 ± 8.7
< 15 ng/ml		61.2%	33.3%	36.1%	30.6%
Body Mass Index (kg/m^2)	187	16.9 ± 2.7	17.4 ± 2.8	16.7 ± 2.8	16.5 ± 2.3
Overweight		19.8%	44.1%	32.4%	23.5%
Obese		19.8%	40.0%	34.3%	25.7%
Oxidative stress measures[2]					
Urinary isoprostane (ng/ml)	186	1.5 ± 1.2	1.4 ± 1.3	1.4 ± 1.1	1.7 ± 1.2
Urinary 8-OH-dG (ng/ml)	188	48.8 ± 33.1	51.3 ± 35.2	48.0 ± 33.5	47.2 ± 30.4
Socio-demographic & parental characteristics					
Mother's education	183				
Some primary		20.2%	33.3%	27.3%	39.4%
Some secondary		65.6%	25.3%	41.4%	33.3%
College or post-graduate		14.2%	55.6%	33.3%	22.2%
Mother unemployed/stay at home	171	39.8%	30.5%	30.5%	39.0%
Mother smoked	181	34.8%	27.6%	34.5%	37.9%
Parent(s) involved in jobs with potential for metal exposure[3]	194	33.1%	27.6%	37.9%	34.5%
Fathers with job exposure risk[4]	131	51.8%	27.4%	30.1%	42.5%***
Family possession of household items[5]	184	8.3 ± 1.9	8.8 ± 1.7	8.0 ± 2.1	8.2 ± 1.9

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			Blood lead concentration, µg/dL		
		All Subjects	***Tertile 1***	***Tertile 2***	***Tertile 3***
Variables			**0.8 – 3.2 (n= 62)**	**3.3 – 4.9 (n= 65)**	**5.0 – 13.2 (n= 55)**
	n	**Mean ± SD or %**	**Mean ± SD or %**	**Mean ± SD or %**	**Mean ± SD or %**
Families do not own a home	154	36.4%	34.4%	32.8%	32.8%
Families with more than 2 person per room	155	22.6%	21.6%	32.4%	45.9%**
Dietary intake					
Energy (Kcal/day)	182	2279 ± 602	2206 ± 593	2300 ± 594	2308 ± 618
Zinc (mg/day)	182	5.3 ± 2.4	5.3 ± 2.8	5.3 ± 2.4	5.3 ± 2.3
< RDA (4 mg/day)[6]		34.2%	35.6%	35.6%	28.8%
Vitamin C (mg/day)	182	59.3 ± 53.6	51.3 ± 54.3	66.4 ± 51.8	58.0 ± 54.0
< RDA (25 mg/day)[6]		19.3%	34.4%	34.4%	31.2%

[1] values adjusted for CRP concentrations

[2] values adjusted for specific gravity (average: 1.023)

[3] has exposure risk if employed in jobs such as construction, factory work, print shop, mechanic and driver

[4] involvement in activities that require handling of chemicals or metals, recycling, auto-repair, plumbing and paint work for 6 months or more; score derived by adding all the responses (0= none of the parents, 1= one of the parents, 2= both parents involved); no involvement if score=0 and involvement if score ≥1

[5] items include TV, video player, DVD player, computer, video games, radio, sound equipment, refrigerator, washing machine, home phone, cellular phone and car

[6] RDA: Recommended dietary allowance set by US-Institute of Medicine

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Table 2

Association between BLL, intakes of vitamin C, zinc and oxidative stress measures[1]

Models	Oxidative stress measures			
	Isoprostane (ng/ml)		8-OH-dG (ng/ml)	
	Unadjusted β [95% CI][2]	Adjusted β [95% CI][3,4]	Unadjusted β [95% CI][2]	Adjusted β [95% CI][3,4]
Model 1- BLL				
BLL, μg/dL	0.08 [−0.001, 0.16]*	0.09 [−0.01, 0.19]*	−0.15 [−2.42, 2.11]	1.16 [−1.64, 3.62]
Model 2- vitamin C and zinc intake[5]				
Vitamin C, mg/d				
1st tertile	Ref	Ref	Ref	Ref
2nd tertile	0.02 [−0.42, 0.46]	−0.26 [−0.74, 0.21]	7.90 [−4.91, 20.71]	−0.79 [−14.36, 12.77]
3rd tertile	−0.15 [−0.59, 0.28]	−0.23[−0.69, 0.23]	−1.44 [−14.04, 11.15]	0.67 [−12.68, 14.02]
Zinc, mg/d				
1st tertile	Ref	Ref	Ref	Ref
2nd tertile	−0.09 [−0.53, 0.34]	−0.12 [−0.61, 0.37]	−3.85 [−17.16, 9.46]	−2.54 [−15.92, 10.84]
3rd tertile	−0.11 [−0.55, 0.33]	−0.21 [−0.71, 0.29]	−16.39 [−29.21, − 3.58]***	−13.51 [−27.18, 1.88]*
Model 3- main effects: BLL & vitamin C[6]				
BLL	0.09 [0.01, 0.18]**	0.10 [0.01, 0.19]**	0.24 [−2.54, 2.06]	0.37 [−2.26, 3.01]
Vitamin C				
1st tertile	Ref	Ref	Ref	Ref
2nd tertile	−0.06 [−0.50, 0.37]	−0.27[−0.73, 0.19]	3.85 [−8.47, 6.18]	2.25 [−7.85, 9.33]
3rd tertile	−0.25 [−0.68, 0.19]	−0.32[−0.77, 0.12]	0.45 [−11.63, 2.45]	3.16 [−4.27, 14.42]
Model 4- main effects: BLL & zinc[7]				
BLL	0.09 [0.01, 0.17]**	0.09 [0.01, 0.18]**	−0.16 [−2.43, 2.11]	0.26 [−2.33, 2.86]
Zinc				
1st tertile	Ref	Ref	Ref	Ref
2nd tertile	0.01 [−0.43, 0.44]	0.20 [−0.27, 0.66]	−2.80 [−15.22, 9.61]	−2.37 [−13.24, 11.26]
3rd tertile	−0.02 [−0.46, 0.41]	0.10 [−0.37, 0.56]	−17.12 [−29.31, − 4.90]***	−14.15 [−23.31, − 0.67]**

[1] complete-case analysis

[2] results from univariate regression models. Model 1: n= 173 (isoprostane), n= 175 (8-OH-dG); Models 2–4: n= 164 (isoprostane), n= 166 (8-OH-dG)

[3] results from multivariate regression models. Model 1: n= 139 (isoprostane), n= 141 (8-OH-dG). Models 2–4: n= 133 (isoprostane), n= 135 (8-OH-dG)

[4] models adjusted for family possession of luxury items (≤8 vs >8 items), parental involvement of jobs with potential for metal exposure (not involved vs involved), crowding at home (no. of people/room), mother's smoking (no vs yes), BMI (kg/m^2) and CRP-adjusted ferritin concentration (ng/mL)

[5] values adjusted for total energy intake by residual method (Willett et a., 1997)

[6] interactions between BLL and vit C were not significant. Models were rerun to test the main effects of BLL and vit C

[7] interactions between BLL and zinc were not significant. Models were rerun to test the main effects of BLL and zinc

*** $p < 0.01$

** $p < 0.05$

* $p < 0.1$

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Table 3

Covariate-adjusted association between BLL, oxidative stress measures and intakes of vitamin C and zinc using multiple imputed dataset[a,b]

Models	Oxidative stress measures	
	Isoprostane (ng/ml) β [95% CI]	**8-OH-dG(ng/ml) β [95% CI]**
Model 1- BLL		
BLL (μg/dL)	0.05 [−0.04, 0.12]	0.21 [−3.24, 2.83]
Model 2- vitamin C and zinc intake		
Vitamin C		
1st tertile	Reference	Reference
2nd tertile	0.03 [−0.41, 0.47]	6.30 [−7.57, 20.17]
3rd tertile	−0.08[−0.51, 0.34]	1.30 [−12.08, 14.70]
Zinc		
1st tertile	Reference	Reference
2nd tertile	−0.08 [−0.50, 0.34]	−1.30 [−14.61, 12.01]
3rd tertile	−0.14 [−0.01, 0.46]	−14.84 [−28.42, −1.26]**
Model 3- main effects: BLL & vitamin C[c]		
BLL	0.05 [−0.01, 0.12]*	0.35 [−3.30, 2.73]
Vitamin C		
1st tertile	Reference	Reference
2nd tertile	0.06 [−0.38, 0.50]	9.36 [−4.57, 23.30]
3rd tertile	−0.14 [−0.58, 0.30]	3.29 [−10.21, 16.80]
Model 4- main effects: BLL & zinc[c]		
BLL	0.05 [−0.03, 0.14]*	0.61 [−3.57 2.35]
Zinc		
1st tertile	Reference	Reference
2nd tertile	0.02 [−0.43, 0.47]	−1.41 [−14.82, 11.99]
3rd tertile	−0.03 [−0.48, 0.43]	−14.89 [−27.83, −1.95]**

[a] number of imputated dataset= 50

[b] models adjusted for family possession of luxury items (≤8 vs >8 items), parental involvement of jobs with potential for metal exposure (not involved vs involved), crowding at home (no. of people/room), mother's smoking (no vs yes), BMI (kg/m^2) and CRP-adjusted ferritin concentration (ng/mL)

[c] interaction between BLL and vit C or BLLand zinc not significant. Models were rerun to test the main effects of BLL and dietary intakes of vit C and zinc

** $p < 0.05$

* $p < 0.1$