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ASSOCIATIONS BETWEEN MICRONUTRIENT INTAKE AND BLOOD LEAD LEVEL IN URUGUAYAN CHILDREN

A Thesis in

Nutrition

by

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ABSTRACT

Background:

Lead exposure is one of the important pediatric environmental health problems in both developing and developed countries. The primary site for absorption of lead is the gastrointestinal tract. Lead is thought to compete with nutrient metals like iron, calcium and zinc for similar binding sites on intestinal transporter proteins for absorption. On the other hand, vitamin C may chelate and excrete lead in urine. It may also alter iron-lead interaction by increasing iron absorption. In addition, there is some evidence that folate metabolism may be related to lead toxicity. Overall, some evidence exists for an association between micronutrient status and lead exposure, but supplementation with micronutrients has not always been effective. It is important to identify a population at risk for both micronutrient deficiency and lead exposure to carry out successful dietary intervention. This cross-sectional study examined the relation between dietary intakes of micronutrients (calcium, iron, zinc, vitamin C and folate) and blood lead level (BLL) in 6-8 year old children (n=61) from Montevideo, Uruguay.

Method:

Sixty-one first graders from four primary schools in Montevideo (the capital of Uruguay) participated in the study. The study children came from low-income families, living in areas known for lead exposure. Fasting blood samples were collected during a clinic visit in school to analyze whole blood lead levels by atomic absorption spectrophotometer. Two 24-hour dietary recalls were conducted with mothers or caregivers and daily nutrient intakes calculated from a database of nutrient composition of Uruguayan foods. Parents filled out a brief questionnaire on

socio-economic status, parental education and employment and child's health. In addition, questions were asked to identify sources of lead exposure. Nutrient intakes were adjusted for total calorie intake by residual analysis. The relationship between micronutrient intakes and BLL were modeled in co-variate adjusted multiple linear and logistic models, with each nutrient tested in separate models and entered as tertiles.

Results:

Mean (SD) BLL of 61 study children was 5.0 (2.6) µg/dL, with 8.2% and 37.7% of the study children having BLL ≥ 10 µg/dL and BLL > 5 µg/dL, respectively. Median (IQR) calorie-adjusted intake of calcium, iron, zinc, vitamin C and folate was 728 (315) mg/day, 10 (5) mg/day, 5 (3) mg/day, 44 (37) mg/day and 325 (240) µg/day, respectively. About 60% children had calcium intake below the Estimated Average Requirement (EAR) (800 mg/day). Intakes of rest of the micronutrients (iron, zinc, vitamin C and folate) were adequate. Nutrient intakes were positively correlated with total daily calorie intake and iron intake was correlated with intakes of vitamin C. Higher dietary calcium intake was associated with lower BLL after adjusting for child's BMI, environmental exposure, family possessions and school. Children in the highest tertile of calcium intake (871-1474 mg/day) had 1.2 µg/dL lower BLL ($p < 0.1$) and 83% lower likelihood ($p < 0.05$) of having BLL > 5 µg/dL than children in the lowest tertile (246 – 728 mg/day). No significant associations were found between BLL and other four micronutrients. There were no significant interactions between iron and calcium or iron and vitamin C.

Conclusions:

Higher calcium intake was associated with lower blood lead level in 58 Uruguayan school children. However, no association was found between intakes of iron, zinc, vitamin C and folate with children's BLL. Future studies should confirm the findings by repeating the results with biomarkers of micronutrients. In addition, the effects of calcium supplementation on BLL in young children are also warranted.

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Chapter 1

INTRODUCTION

Metal pollution and its harmful effects on health are increasing globally. For example, the toxicity caused by metals has been described as a silent epidemic (CDC 1991; Grandjean et al. 2006). Lead, one of the heavy metals, performs no useful biochemical function in the body, but it can exert a wide range of toxic effects (Goyer RA. 1993). Environmental lead exposure is a major public health problem both in developing and developed countries (Tong et al. 2000). The main sources of lead exposure include emissions from vehicles that use leaded fuels, water supplied through leaded pipes, lead-based paint in older housing, lead in soil and dust from contaminated leaded paint and gasoline, food prepared in lead-glazed ceramics, and industrial activities. While lead has been withdrawn from gasoline in most countries around the world, exposure from other sources still continues (CDC 2005).

The primary site of absorption of lead is the gastrointestinal tract. Absorption of inorganic lead occurs primarily in the duodenum (Mushak 1991). The exact mechanisms of absorption are not clear but it is thought that lead absorption may involve active transport and/or passive diffusion (ATSDR 2007). Since lead is a divalent cation, it is thought that carrier-mediated saturable transport mechanisms for lead may exist within the mucosal and serosal membranes and within the intestinal epithelial cells (ATSDR 2007). For example, lead is thought to compete with iron on the iron binding protein DMT1 (Divalent Metal Transporter 1) for intestinal absorption (Bannon et al. 2002). Lead also competes for similar binding sites on intestinal proteins that are used by calcium for absorption (Barton et al. 1978a; Bronner et al 1986; Fleming et al 1998b; Gross and Kumar

1990; Teichmann and Stremmel 1990). Increased levels of 1, 25 Dihydroxycholecalciferol, the active form of vitamin D, stimulate the intestinal absorption of lead by stimulating the synthesis of calcium binding protein calbindin (Peraza et al 1998; Edelstein et al 1984). This is similar to the effects of cholecalciferol on calcium absorption (Bronner et al. 1986; 10, Fullmar and Rosen 1990). Lead-zinc interaction at the intestinal cells also may influence absorption of lead as lead may compete with zinc for similar binding site (Peraza et al 1998; ATSDR 2007).

Infants and young children are at high risk of exposure through frequent hand-to-mouth activity (Lanphear et al 2002). Children absorb up to 50% of the lead they ingest, about five times as much as adults (Bearer, 1995; ATSDR 2007). Lead is also absorbed rapidly through the lungs when inhaled (ATSDR 2007; Hursh et al. 1969, Chamberlain et al 1975, Wells et al 1975) and through the skin (ATSDR 2007; Rastogi, 1976). Once absorbed, lead is distributed in blood, soft tissues such as liver, kidney, lungs, spleen, muscle, heart and brain; and mineralizing tissues such as teeth and bones (ATSDR 2007). While the blood lead level (BLL) in children reflects recent or current exposure, lead in the bones can be stored for decades and mobilized into the circulation during conditions such as pregnancy, lactation and osteoporosis (Hu, H 1998).

Adverse health effects of lead:

Lead can affect almost every organ system in the body (ATSDR 2007). The adverse effects of lead in children depend on the dose, age and duration of exposure (CDC 2005). While overt lead poisoning is not as common as just three decades ago (Needleman, 2004), our main concern today is with populations exposed to lead in amounts that do not result in overt signs or symptoms of poisoning but that still have important effects on child development. The permeable blood-brain barrier, along with the developing nervous system makes young children especially susceptible to lead neurotoxicity (Bellinger, 2008). Although, the level of concern established by the Centers of

Disease Control (CDC) is currently set at 10 µg/dL, research continues to show inverse association between lead exposure and children's intelligence even at blood lead level below 10 µg/dL (Canfield et al. 2003; Chiodo et al. 2004; Lanphear et al. 2005; Lanphear et al. 2000; Kordas et al. 2006).

Effect of lead on cognition and behavior:

Recent studies find negative associations between lead exposure and cognitive impairments at concurrent blood lead concentrations below10 µg/dL in school age children even after controlling for potential confounders (Chen et al 2005 32; Lanphear et al 2005; Wasserman et al 2003; Kordas et al 2006). Lead-associated deficits have been reported in most domains of cognitive function including verbal IQ, performance IQ, academic skills such as reading, math and spelling, visual/ spatial skills, problem solving skills, executive functions, fine and gross motor skills, memory and attention in children with BLL < 10 µg/dL (Bellinger, 2008). In a pooled analysis of data from 7 international longitudinal cohorts, Lanphear et al (2005) observed a 6.2-point decline in IQ for an increase in BLLs from < 1 to 10 µg/dL in children who were followed from birth or infancy until 5-10 years of age. Previously, in a prospective longitudinal analysis, Canfield et al (2003) found an average loss of 7.4 points on the Stanford- Binet intelligence test over the first 10 µg/dL of lifetime average BLL. Jusko et al (2008) found that 6 year old children with a mean BLL < 5 µg/dL scored 4.9 IQ points higher on the Wechsler Intelligence Scale than children with a mean BLL between 5 and 9.9 µg/dL. In addition to IQ, using data from NHANES III, Lanphear et al (2000) examined measures of non-verbal reasoning, memory and achievement in relation to concurrent blood lead concentrations in US children aged 6-16 years. Significant inverse associations were found between BLL below 10 µg/dL and memory, visual spatial

abilities, arithmetic and reading. Moreover, BLL was significantly and inversely related to reading and arithmetic scores even in children with BLL < 5 µg/dL.

Evidence also suggests a non linear relationship between lead and cognitive deficits (Kordas et al 2006; Chiodo et al 2004; Canfield et al 2003; Lanphear et al 2000), where the rate of decline in IQ scores appears to be greater at lower blood lead levels. For example, in Mexican 1st graders, Kordas et al. (2006) observed that cognitive deficits for each 1µg/dL increase in the BLL were greater across the range of < 10-14 µg/dL, than above this range. Moreover, from the international pooled analysis of longitudinal studies, Lanphear et al (2005) estimated that IQ decrements associated with an increase in blood lead level from 2.4 to 10 µg/dL, 10 to 20 µg/dL and 20 to 30 µg/dL were 3.9, 1.9 and 1.1 points respectively, suggesting that the effects of an increase in blood lead concentrations are greater in children with BLL < 10 µg/dL compared to children with BLL >10 µg/dL.

Behavioral deficits have also been shown in children with low level lead exposure. Thomson et al (1989) reported a significant relationship between children's blood lead and total scores on the teacher's rated Rutter behavior questionnaire and the aggressive/ antisocial and hyperactive sub-scores in 6-9 year old children with a mean BLL of 10.4 µg/dL. Recent studies have found a wide range of behavioral problems such as inattention (Bellinger et al 1994; Chiodo et al 2007; Roy et al 2009), disorganization (Bellinger, 2004), hyperactivity (David et al 1972; Needleman et al 1979; Lansdown et al 1986; Kordas et al 2005), executive function (Roy et al 2009; Lanphear et al 2000; Canfield et al 2004) and off-task behaviors (Kordas et al 2007; Chiodo et al 2004) related to childhood lead exposure, with no evidence of a threshold. Braun et al (2006) analyzed the data from 4,704 children (4-15 years old) from NHANES III and found that the risk of parent- reported diagnosis of ADHD behavior increased, in a dose-dependent manner, with

higher blood lead level. The association between children's blood lead and ADHD remained significant even when the analysis was restricted to BLL < 5 µg/dL. Finally, low level lead exposure in early childhood has been linked with delinquent behavior (Needleman et al 1996; Dietrich et al. 2001) and the development of criminal activities and anti-social behaviors in adolescence (Wright et al. 2008; Nevin, 2007; McCall et al. 2004).

Other health effects of lead:

In addition to the neurotoxic effects, lead exposure even at lower levels is associated with decreased growth (Schwartz et al 1986; Shukla et al 1989; Kafourou et al 1997), decreased hearing acuity (Schwartz and Otto 1987) and higher incidence of dental caries (Moss et al 1999; Campbell et al 2000; Gemmel et al 2002) in children. For example, Ignasiak et al (2006) found a significant relation between blood lead level and reduced weight, height, trunk, leg and arm lengths in 899 Polish school children with a mean BLL < 10 µg/dL. Schwartz et al (1991) found that blood lead level was positively associated with hearing loss at 500, 1000, 2000, and 4000 Hz, with no evidence of a threshold. Moreover, a 5 µg/dL change in BLL was associated with an elevated risk of dental caries in 5-7 year old US children who participated in NHANES III, even after adjustment for socio-demographic characteristics, diet and dental care (Moss et al 1999). This growing evidence of adverse effects of low level lead exposure particularly on children's cognition, behavior and school performance has shown that there is no "safe" level of lead in blood and underscores the continuing need to reduce lead exposures even after preschool years (CDC, 2001).

Nutritional status and lead exposure:

Because ingestion of lead is the primary route of exposure in children, the study of relationships between dietary nutrients and lead absorption and toxicity has been an active area of

research (CDC 2001). Indeed, both animal and human studies have shown that nutrition plays an important role in lead absorption and toxicity (Mahaffey et al 1990). Many lead exposed populations are also at risk of nutritional deficiencies (CDC 2001). Undernourishment and particularly deficiencies of micronutrients may affect lead levels in the body. Children's high rate of growth and development creates windows of vulnerability to both nutritional deficiencies and lead exposure. Accumulating evidence suggest that there are several ways that nutrition and environmental chemicals such as lead are interconnected (Mahaffey et al 1990; Kordas et al 2007b). First, food consumption and frequency of food intake influence absorption of lead from the gastrointestinal tract (Peraza et al 1998; ATSDR 2007), with lead being absorbed to a much greater extent during fasting. Rabinowitz et al (1980) reported that among adult male subjects, the gastrointestinal lead absorption increased when lead was ingested in the absence of food. Similar results have been reported by others (Blake et al 1983; Flagan et al 1982).

Second, nutritional status of children may affect the susceptibility to lead exposure and subsequent toxicity. Evidence exists for interactions between lead and micronutrients at the level of intestinal absorption, metabolism and sites of action in the body (Kordas et al 2007b; Pereza et al 1998; Mahaffey, 1995). Dietary nutrients potentially interact with lead by binding it in the gut, competing with lead for absorption, altering cell avidity for lead or altering the affinity of the target tissues to lead, as described below.

Iron and lead

Iron and lead share the Divalent Metal Transporter 1(DMT1) for intestinal absorption (Bannon et al 2002). Experimental studies have demonstrated that iron-deficient (ID) animals absorb greater percentage of ingested lead than iron-replete animals (Six and Goyer, 1972; Barton et al 1978). Evidence from human studies also has shown that ID is associated with elevated BLL

both in young children (Wright et al 1999) and adults (Graziano et al 1990). Wright et al. (2003) conducted a longitudinal study of 1275 children who were followed over a 3-year period (age range 9-42 months) and found that baseline iron-deficiency was associated with elevated blood lead level over time. Dietary iron intake was also inversely associated with blood lead level among pre-school children (Hammad et al 1996). In another longitudinal study with 3-12 month old infants, after adjustment for confounding covariates, low iron intake was significantly associated with higher BLL both at 6 months and 12 months of age (Schell et al 2004). Placental transfer of lead was lower among women who had higher hemoglobin levels (Harville et al 2005). Maternal dietary intake of iron during pregnancy was also negatively associated with neonatal blood lead level (Schell et al 2003). In a cross sectional examination of nutritional factors and blood and bone lead levels among 747 men aged 49-93 years, who participated in the Normative Aging Study, an inverse association was observed between BLLs and total dietary intake of iron after adjusting for patella lead, age, smoking and alcohol consumption (Cheng et al 1998). In well nourished Mexican first graders low serum ferritin level was more prevalent in children with BLLs above 15 µg/dL than below this level (Kordas et al 2004). Conversely, other cross sectional studies found no association between ID and blood lead level (Wolf et al 1994; Campbell et al 2000). Iron supplementation has so far provided mixed results, with some studies showing positive effects (Wolf et al. 2003; Zimmerman et al 2006), while others reporting no effects of iron supplementation on lowering children's BLLs (Rosado et al 2006). Current knowledge about iron and BLL is conflicting; with some studies reporting negative associations while others finding no associations. Moreover, most of the studies have used iron biomarkers (such as hemoglobin, serum ferritin) to examine the association between iron and lead, and there is a lack of studies on dietary

iron intake and blood lead level particularly in school age children. Knowledge of the association would help to implement a successful intervention.

Calcium and lead

Compared to other nutrients, a large body of literature exists on the relationship between dietary calcium and BLLs both in animals and humans. Evidence suggests that low ingestion of calcium increases lead absorption and toxicity. Animals maintained on low-calcium diets have higher intestinal lead absorption (Barton et al 1978; Six and Goyer 1970; Quarterman and Morrison1975; Heard and Chamberlain 1982) and higher whole body or organ contents of lead in response to chronic lead exposure than animals on control diets with adequate calcium content (Six and Goyer 1970; Barltop and Khoo 1976; Bogden et al 1992). In humans, increasing dietary calcium was associated with decreases in gastrointestinal lead absorption and BLL in some studies (Ziegler et al. 1978; Sorrel 1977; Mahaffey et al 1986; Bogden et al 1992; Quarterman et al 1978) but not in others (Rosen et al 1980; Lanphear et al 2002). A study of 2,926 US children from Second National Health and Nutrition Examination Survey (NHANES II) showed that dietary calcium intake measured by a 24 hour dietary recall was inversely associated with blood lead level in 1-11 year olds with a mean BLL of 15.7 µg/dL after controlling for confounding factors (Mahaffey et al 1986). Moreover, higher calcium intake was associated with lower BLLs in pregnant women (Hertz et al 2000). Calcium supplementation has produced modest reductions in blood lead when administered both during pregnancy (Ettinger et al 2009; Téllez-Rojo et al 2006) and lactation (Hernández et al 2003; Ettinger et al. 2006). It is thought that lead competes with calcium at calcium-binding sites and may subsequently alter protein function and calcium homeostasis (Sauk and Somerman, 1991). However, Hammad et al (1996) and Lucas et al (1996) examined 299 children of low-socioeconomic status aged 9-72 months and did not find significant

associations between dietary calcium intake, serum calcium concentration and BLL in multivariate models controlling for serum iron indices and for dietary protein, fat, fiber, phosphorous, potassium, total energy, carbohydrate, thiamin, and vitamin C. Supplementation with calcium also has not provided conclusive results in children. In a randomized clinical trial, no measurable effect of calcium supplementation was found on infant's blood lead level (Sargent et al 1999). Similarly, in a double-blind placebo controlled trial, Markowitz et al (2004) did not find a benefit of calcium supplementation on blood level in 1-6 years old children who were already calcium sufficient. The average daily calcium intake of children in both placebo and supplemented groups in this study was greater than the recommended daily intake (800 mg) for this age-group at enrollment. Although BLL decreased in both groups over the study period, calcium supplementation aimed at providing 1800 mg of Ca/day had no effect on the change in BLLs. Additional studies are required to confirm the association between calcium intake and BLLs in children.

Zinc and lead

Interactions between zinc and lead have been investigated at absorptive and enzymatic sites (Flora et al 1982). A negative association between zinc and lead has been found in experimental animal studies (Bushnell and Levin 1983; Flora et al 1989; Petering 1978). Zinc and lead compete for similar binding sites on the metallothionein- like transport protein in the gastrointestinal tract and zinc treatment reduced lead concentrations in kidneys and lungs in rats (Kumar et al 1991). Zinc was also shown to have an antagonistic effect against lead induced inhibition of δ-aminolevulinic acid dehydratase (δ-ALAD), an enzyme in the heme-synthesis pathway, both in animal and human studies (Haeger and Schutz 1976; Dutkiewicz et al 1979; Flora et al 1989). Moreover, among limited human studies that have addressed the zinc-lead relation, Schell et al (2004) found an inverse association between dietary zinc intake and BLL in 6 month old infants.

Zinc supplementation in animals has been shown to reduce tissue accumulation and toxicity of lead (Batra et al.1998; Cerklewski and Forbes 1976; Jamieson et al 2008). In addition, in an experimental study, administration of zinc along with calcium disodium EDTA increased the chelation and excretion of lead in rats (Flora et al 1994). However, in a randomized double-blind placebo controlled trial Rosado et al (2006) did not find any effect of zinc supplementation on BLLs of school age children, similarly with another study conducted in adults (Lauwerys et al. 1983). Since there is a lack of studies in school age children, examination of the relation between dietary zinc intake and BLL is important for future interventions.

Vitamin C and lead

Ascorbic acid or vitamin C is another micronutrient that is associated with blood lead level. Evidence from animal studies (Dhawan et al 1988; Goyer and Cherian, 1979) suggests an antagonistic effect of vitamin C on lead absorption and body metabolism, with orally administered vitamin C possibly having similar chelating properties as EDTA (ethylenediaminetetraacetic acid). Based on these animal studies, one possible protective mechanism may be the chelation of lead by vitamin C and excretion through urine. However, there are not many human studies to support this hypothesis. Results of a single-blind clinical trial of vitamin C among lead smelter workers with BLLs of 28 to 76 µg/dL showed no effect of vitamin C on urinary lead excretion (Lauwyers et al 1983). In a double-blind randomized clinical trial, however, adult male smokers given a daily dose of vitamin C experienced a statistically significant 80% decline in blood lead level (Dawson et al 1999). In a population based study from NHANES III, serum vitamin C level was inversely associated with the prevalence of elevated blood lead concentrations in both youths (6-16 years) and adults (Simon and Hudes, 1999). In this study, there was no significant relationship between dietary vitamin C intake and blood lead levels. However, Cheng et al. (1998) observed inverse

associations of blood lead levels with total dietary intake of vitamin C in adult men. When analyses were controlled for patella lead, age, smoking, and alcohol consumption, men in the lowest vitamin C intake quintile (< 109 mg/day) had a mean blood lead level 1.7 μg/dL higher than men in the highest quintile (≥ 339 mg/day).

Vitamin C can also play a key role in the relationship between iron and lead, since it is well known that vitamin C increases dietary iron absorption (Moore and Dubach 1951; Cook and Monsen 1977; Hallberg et al. 1986). One animal study has shown that dietary supplementation with iron and ascorbic acid prevented the growth retardation, anemia and accumulation of lead in the kidneys of rats (Suzuki and Yoshida 1979). But how vitamin C affects the iron-lead interaction and subsequently lead absorption is not well understood. Moreover, the effect of vitamin C on blood lead level in children is not clearly understood. Further research into the relationship between vitamin C (dietary intake and blood level) and its involvement in lead absorption and accumulation in humans is required. The knowledge of an association, if any, would be important to correct ID and reduce BLL.

Folate and lead

There is some evidence that folate metabolism may be related to lead toxicity. Lee et al. (2005) reported an inverse association between serum folate levels and BLLs in US women of reproductive age. In contrast, the relationship between dietary folate intake and blood lead level is not well understood. In one study, supplementation with folate enhanced the urinary excretion of lead, mobilized tissue lead and restored lead-induced biological alteration in lead intoxicated rats (Tandon et al 1987). Studies on folate status and blood lead level in children are limited. A recent study from the Philippines suggests that higher erythrocyte folate concentration may attenuate the

adverse relationship between lead exposure and cognitive performance in children (Solon et al 2008). Together these data suggest the importance of research on folate status and lead exposure in children.

Summary:

Lead exposure continues to be a major pediatric health problem in both developing and developed countries. A growing body of literature exists on the neurotoxicity caused by lead even at very low level of exposure. Although removal of lead from children's environment remains the primary means of preventing elevated blood lead levels, reduction of risk through nutritional intervention has also been of interest. However, there are gaps in knowledge about the relationships between children's blood lead levels and specific nutrients that would allow the establishment of specific recommendations for dietary intervention (CDC 2001). Moreover, because there is an overlap between malnutrition and lead exposure, it is important to determine associations between the status and intakes of specific nutrient and susceptibility to lead exposure and subsequent toxicity in children, particularly at low-level exposure. This in turn, may help prevent lead exposure and alleviate nutrient deficiencies as well. This study examined the relationship between dietary intakes of micronutrients with lead levels among school children aged 6-8 year olds from Montevideo, Uruguay. The primary objective of this study was to examine the association between the dietary intakes of selected micronutrients and blood lead levels among school children aged 6-8 years from Montevideo, Uruguay.

Specific aims were to

1) Examine the demographic, family and child characteristics related to blood lead level in this population.

2) Determine whether dietary intakes of micronutrients- calcium, iron, zinc, vitamin C and folate were related to children's blood lead levels.

3) Examine the effects of micronutrient interactions (Calcium-Iron, Iron-vitamin C) on blood lead levels of children.

Chapter 2

METHODS AND STATISTICAL ANALYSIS

Study settings and participants:

This cross sectional study was carried out in 61 school children (6-8 years old) of low to middle income families attending 1st grade in Montevideo, the capital city of Uruguay. The population of Montevideo is 1.9 million as estimated in 2009 (Instituto Nacional de Estadistica, 2009). Unlike other Latin American countries, Uruguay is not a lead producer. But lead is imported for industrial use (Romieu et al. 1997) and Montevideo has several lead emitting industries, many of them located in residential areas (Cousillas et al 2005). In addition, leaded gasoline was in use until 2004 and much of the city's drinking water system still uses leaded pipes. Previous studies have shown that lead exposure is prevalent among Uruguayan children (Cousillas et al. 2005). Furthermore, a blood lead and hemoglobin screening by Queirolo et al (2010) in 222 preschool children aged 5-45 months from several areas of Montevideo indicated a considerable overlap between lead exposure and anemia with 32.9% children having BLL $\geq$ 10 ug/dL, about 72% with BLL $\geq$ 5 ug/dL and 44.1% being classified as anemic (hemogloboin <10.5 g/dL). In this same study, anemia was a strong predictor of children having BLL > 10 ug/dL.

The study of first-graders took place from July 2009 to July 2011. Primary schools from several neighborhoods around Montevideo were invited for participation in the study after meetings with the directors of the schools. Upon acceptance by the school authorities, posters and leaflets were distributed in schools to invite parents of all 1st graders for an informational meeting. General information on childhood lead exposure: common sources, route of exposure, absorption, health effects, and specific information about the study procedure were provided during these

meetings. Parental consent was obtained during these informational meetings at the school or parents returned the signed consent forms after consultation with their partners and other family members. In addition, an oral assent from the child was obtained at the time of blood collection. Children were enrolled in the study if they did not have previous history of lead poisoning (BLL > 45 ug/dL). The study was approved by the Biomedical Institutional Review Board of the Pennsylvania State University and Human Research Ethical Committees of Catholic University and the University of Republic of Uruguay.

Assessments:

Biochemical measurements:

Fasting blood was collected by a phlebotomy nurse in a clean room at the schools, during a morning visit between 8 and 11 am. A parent or an adult accompanied the child during the blood draw. Approximately 3 ml of venous blood was collected from each child in heparin vacutainer trace-metal free tubes (Becton Dickinson, Franklin Lakes, NJ USA) for lead analysis. If possible, additional 3 ml of venous blood was also drawn in 2 separate vacutainer tubes for serum and plasma separation, respectively. Hemoglobin was also measured in a few drops of venous blood, removed from one of the tubes, using a portable Hemocue hemoglobinometer (HemoCue Inc, Lake Forest, CA). Quality control was performed with standard HemoCue controls every day. Children were provided with light breakfast afterwards consisting of cakes or biscuits and yogurt.

Serum and plasma were separated by centrifuging at 2000 rpm for 10 min. Approximately 250 µl of serum were aliquoted for serum ferritin (SF) and C-reactive protein measurement. Serum samples were stored on ice in a cooler and transported to the laboratory within one hour of completion of the clinic. At the laboratory, serum samples were stored at -20°C for future analysis.

Serum samples were shipped on dry ice to the laboratory at Pennsylvania State University where serum ferritin was analyzed in triplicate with a CV of 5% using a radioimmuno assay technique (Coat-a-Count ferritin IRMA, Siemens Health Care Ltd, USA).

Blood lead was analyzed at the Toxicology Laboratory of the University of Republic (Montevideo, Uruguay) using the flame atomic absorption spectrophotometer (Perkin Elmer 306, Norwalk, CT) with a detection limit of 3 µg/dL. The laboratory participates in monthly quality control programs with the Institute of Workplace Safety and Hygiene in Spain and the CDC.

Anthropometric measurements:

Height of the children was measured in triplicate to the nearest of 0.1 cm, using a stadiometer (Seca 214, Shorr Productions, Colombia, MD) with children without shoes and standing in a steady posture. Children were also weighed with minimal clothing (without sweater or jacket) in triplicate to the nearest 0.1 kg using a digital scale (Seca 872, Shorr Productions, Colombia, MD). Children's weights in triplicate were averaged and final weights were calculated by subtracting the weights of clothing children were wearing at the time of measurement.

Dietary assessments:

To determine children's dietary intakes of total energy and nutrients like carbohydrate, protein, fat, fiber, iron, zinc, calcium, folate and vitamin C, two 24- dietary recalls were conducted by five trained nutritionists with the mother or with the caregivers, one recall took place at the school and another within 2 weeks, over the phone without prior appointment, normally on a weekday. A detailed list of all the foods and beverages the child consumed within the last 24 hour period were 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 name of commercial products. To facilitate food portion recalls, food models and household measurement cups were used during the personal interview with mothers or caregivers. Along with general information, intake of vitamin and mineral supplements 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 coded afterwards and dietary data along with amounts consumed were entered into a database of nutrient composition of Uruguayan foods. Calculations of nutrient intake were derived from these data.

Socio-demographic assessments:

During the informational meetings at school, parents or caregivers filled out questionnaires about family income, possessions and home ownership to determine family's economic status. Information was also obtained on parental education and occupations, crowding at home and year of construction of the house where the child lives. Additional information about children's birth weight and medical history was gathered. To identify possible environmental sources of exposure, children's outside play habits, frequency of dust cleaning at home, sources of drinking and cooking water were gathered. The questionnaires were self-administered but the research staff provided assistance if parents or caregivers asked for clarification while filling out the questionnaire.

STATISTICAL ANALYSIS

Dietary data:

Dietary data from two 24 hour recalls were averaged for each individual nutrient. Where one dietary recall was missing (n=1), daily nutrient intake was calculated from one dietary recall. Spearman correlation coefficients were calculated among the intake of different nutrients. All the nutrient values obtained from averaging two diet recalls were adjusted for calorie intake using the nutrient residual model approach (Willett 1998). Calorie-adjusted nutrient intakes were computed as the residuals from the regression model with total calorie intake as the independent variable and micronutrient intake as the dependent variable (Willett 1998). The rationale behind energy adjustment is that individual differences in total energy intake produce variation in intake of specific nutrients unrelated to dietary composition, and it has been shown that consumption of most nutrients is positively correlated with total energy intake (Willet et al.1997). Scatter plots and distributions of dietary intake of calcium, iron, zinc, vitamin C, folate and total calories were examined to identify outliers. Children with calorie intake more than 3500 Kcal (n=3) were considered as outliers and excluded from further analysis for a final number of 58 children. We decided to use this cut-off since the 95^{th} percentile for calorie intake among our study children was 3469 Kcal/day. Moreover, the 95^{th} percentile for daily calorie intake among children aged 5 to 8 years in NHANES III was 2463 Kcal/day (CDC 2003). A child with an iron intake more than 52 mg (Tolerable upper intake levels for iron for this age group: 40 mg/day) were excluded from further analysis. The daily calorie-adjusted vitamin C intake was reported to range from 0-205 mg/day for the study children. For biological implausibility, children with reported vitamin C intake of 0 mg/day (n=2) were excluded from further analysis of data concerning vitamin C intakes

for a final number of 56 children. Finally, tertiles were created for each nutrient because the intakes of the nutrients were not normally distributed.

Environmental Lead Exposure Index:

Because of correlation among questionnaire items that measured exposure to lead, an index for exposure to lead was constructed, as the sum of responses to the individual questions (Table 1). The index score ranged from 7 to 75. Selection of the index components was based on the possible sources of lead contamination or activities that would increase the probability of lead exposure in this population. In crude analysis not all of the variables included in the index were statistically associated with blood lead.

Table 1: Items used in constituting the environmental exposure index

Items	Score
Mother smokes	
No	0
Yes	1
Parent's job lead exposure risk[a]	
No	0
One parent	1
Both parents	2
Drinking water	
Bottled	0
Filtered tap water	1
Unfiltered tap water	2
Tank	3
Cooking water	
Bottled	0
Filtered tap water	1
Unfiltered tap water	2
Tank	3
Frequency of cleaning the floors	
More than once per week	1
Once a week	2
Less than once a week	3
Frequency of dusting the house	
More than once per week	1
Once a week	2
Less than once a week	3
Members took off shoes outside	
No	1
Yes	0
Families use door-mats	
No	2
Yes	0
Sometimes	1
Child spends playing outside	Number of hours/week

[a] Jobs involving battery recycling, print shop, mechanic, driver, plumber, construction, worker at chemical and paint industry, manufacture of plastic and metals.

Socio-economic status:

Since many parents refused to answer questions on family income or other expenses, a proxy variable of family possessions was created to determine the socio-economic status of the families. Ownership of 12 household items (TV, videocassette, DVD, computer, video games, automobile, radio, music system, refrigerator, washing machine, home phone and cellular phone) were enquired and the positive responses were summed to calculate the total number of items families possessed (Range: 5-12). "Family possessions" was used as a continuous variable in all of the regression models. However, in children for whom family income data was available (n= 31), family possessions were highly correlated with monthly income (spearman $\rho = 0.7$, $p < 0.001$).

Descriptive statistics:

Descriptive statistics were primarily used for the exploratory analysis of the data, with student's t-tests used for continuous variables and chi-square tests for categorical variables. Data were plotted to examine the distributions of blood lead level and micronutrient intakes. The association between micronutrient intakes and blood lead level was investigated with multiple linear and logistic regression models.

Student's t-tests, chi-square tests, and one-way analysis of variance (ANOVA) were used to examine differences between BLL within various demographic and health-related strata. Sex difference in nutrient intakes was examined by Student's t-tests and median tests. The difference in nutrient intake values due to data collection by five interviewers and differences in nutrient intake in different months were tested by ANOVA. Spearman correlation coefficient was used to assess associations between nutrient intakes and BLL within each school. A p-value of < 0.05 was considered statistically significant for all statistical tests.

Selection of covariates:

All the socio-demographic and exposure characteristics were tested in bivariate regression models as potential predictors of BLL. Next, all the variables with $p< 0.1$ were included in the final multivariate models examining the associations between micronutrient intakes and BLL. The following variables were used as covariates in the final models: children's BMI, family possessions, environmental exposure index and school. Because a large proportion of the study children came from the school "Jesus Isaso", school was collapsed into children attending the school "Jesus Isaso" versus other schools. While BMI, family possessions and environmental exposure index were entered as continuous variables, schools were treated as a categorical variable (dichotomized as 0 and 1, with the school "Jesus Isaso" being coded as 1 and others being coded 0).

Multivariate modeling:

Multiple linear regression analysis was performed to examine the association between calcium, iron, zinc, vitamin C, and folate and children's BLL, where BLL was entered as dependent and nutrient intake as independent variable. Separate models were used to test each nutrient. Blood lead level was entered as continuous outcome variable and calcium, iron, zinc, vitamin C and folate intakes as tertiles. Models were adjusted for covariates (BMI, family possessions, environmental exposure and school). Multivariate logistic regression models were used to test the likelihood of having BLL > 5 µg/dL among children in lower and higher tertiles of each micronutrient intakes. We chose a cutoff value of 5 µg/dL over 10 µg/dL, because most of our study children have lower BLLs (< 10 µg/dL) and also because studies continue to show detrimental effects of lead in children at BLL < 5 µg/dL (Jusko et al 2008; Braun et al 2006).

We created interaction terms for calcium-iron by crossing each tertile of calcium with corresponding tertile of iron intake. Thus three interaction terms were used for calcium-iron, with interactions between lowest tertiles of calcium and iron intake being the reference level. Similar, interaction terms were created for iron-vitamin C. These interaction terms were included in the regression models to test whether the interactions affect children's BLL. The interactions were considered significant at p-values less than 0.05 and interactions that were not statistically significant were excluded from the models and the models were re-run with the main effects of nutrients only. All data were analyzed using STATA 8.0 (College Station, TX).

Chapter 3

RESULTS

Participant characteristics:

Figure 1 summarizes the number of subjects eligible, participated and excluded from the study. The general characteristics of the study participants are presented in Table 2. Four primary schools from 2 neighborhoods ("Cerro" and "La Teja") of Montevideo comprised the study site and a total of 61 children attending first grade at the schools completed all the evaluations. The mean (SD) age of the participating children was 6.4 (0.5) years. Study children comprised of more boys (62.3%) than girls (37.7%). Girls were slightly younger than the boys [Mean (SD): 6.2 (0.4) years vs 6.5 (0.6) years; $p< 0.1$]. Almost 45% and 28.8% of the mothers did not have secondary education and did not work outside of the home, respectively. Almost 50% of the families did not own a home. The average family possessions of the items were 8 (of the 12 items queried) and less than 15% of the children lived in households with more than 2 people per room.

The mean (SD) blood lead level (BLL) of the children was 5 (2.6) µg/dL with a range of 2.8 to 12.5 µg/dL (Table 2), with 8.2% of the children having BLL≥ 10 µg/dL and 37.7% having BLL> 5 µg/dL. Among the study children, the majority (95%) did not have any previous blood test for lead. The mean (SD) hemoglobin concentration was 13.6 (1.2) g/dL, with only one child being classified as anemic (hemoglobin< 12 g/dL) for this age group. However, serum ferritin level was low in these children [Mean (SD): 9.2 (6.5) µg/L], with about 77.5% of the study children was iron-deficient (serum ferritin < 15 µg/L). The co-occurrence of elevated BLL (BLL≥ 10 µg/dL) with iron-deficiency was 6.8%. Approximately 16% of the study children were overweight (BMI-for-age ≥ +1 SD to < +2 SD) and 20% were obese (BMI-for-age > +2 SD) according to WHO

2007 reference tables (WHO 2007). Boys were slightly taller than the girls ($p< 0.05$) (Data not shown). However, there were no sex differences in weight and BMI among the study children.

Independent sample t-test revealed that there was no significant sex difference in BLL in the study (Table 2). Overweight children had lower BLL than the normal weight children (3.3 ± 1.0 µg/dL vs 5.6 ± 2.8 µg/dL; $p< 0.05$). Children in the school "Jesus Isaso" had higher mean BLL than children in other schools and children in families with more family possessions had lower BLL than families with fewer items ($p< 0.05$). Significant differences in BLL were not found with other child and family characteristics (Table 2).

Figure 1: Summary of study eligibility, participation and exclusion of children

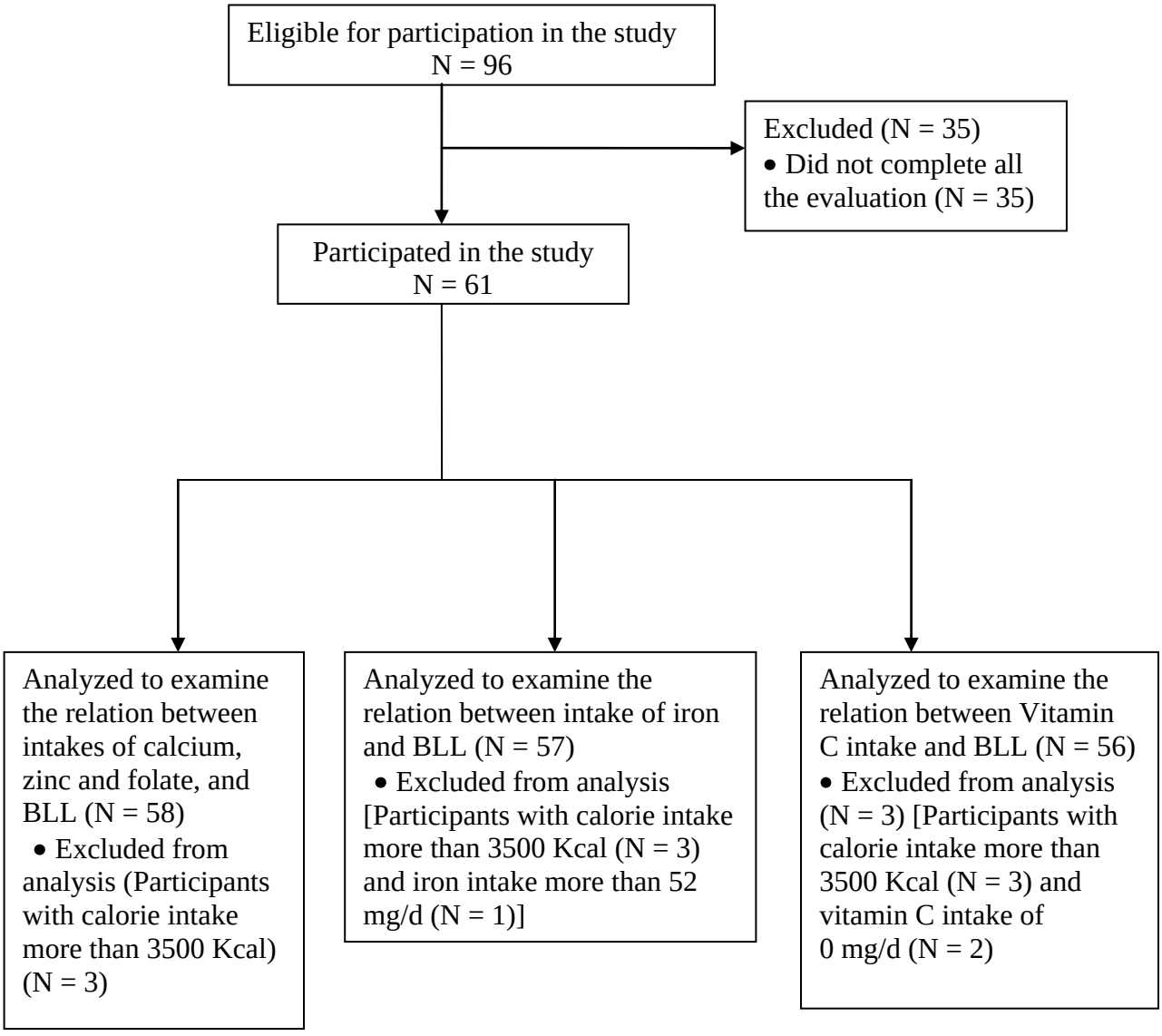

Table 2. Sample characteristics of 61 children participating in the study

Child/Family characteristics	Mean ± SD Or Prevalence	Range	Blood lead (µg/dL)[a]
	Child characteristics		
Child age (year)	6.4 ± 0.5	6 – 8	---
Child sex			
Boys	62.3%	---	5.4 ± 3.2
Girls	37.7%	---	4.8 ± 2.2
Blood lead level(µg/dL)	5.0 ± 2.6	2.8 – 12.5	---
≥ 10.0 µg/dL	8.2%	---	---
> 5.0 µg/dL	37.7%	---	---
Hb (g/dL)	13.6± 1.2	10.5 – 16.3	---
< 12.0 g/dL	1.6%	---	---
Serum ferritin (µg/L)	9.2 ± 6.5	1.2 – 24.6	---
< 15 µg/L	77.5%	---	---
Height (cm)	123 ± 5.8	111 – 138	---
Weight (kg)	25.4 ± 5.4	17.6 – 41.4	---
BMI (kg/m^2)	16.7 ± 2.5	12.8 – 23.0	
Normal weight (> -2 SD to +1 SD)	63.9%	---	5.6 ± 2.8
Overweight (≥ +1SD to < +2 SD)	16.4%	---	3.3 ± 1.0**
Obesity (> +2 SD)	19.7%	---	4.6 ± 2.2
School			
Jesus Isaso	59.0%	---	6.2 ± 2.8**
Other	41.0%	---	3.7 ± 0.9
	Family characteristics		
Mother's education			
Any primary	21.3%	---	5.1 ± 2.2
Any secondary	39.3%	---	5.3 ± 2.7
Any postsecondary	39.4%	---	4.7 ± 2.8
Mother unemployed/stayed at home			
No	71.2%	---	5.3 ± 2.8
Yes	28.8%	---	4.9 ± 2.6
Family's house			
Own	50.8%	---	5.0 ± 2.7
Rented	14.8%	---	5.2 ± 2.9
Shared with family/other	34.4%	---	4.8 ± 2.3
Crowding	1.8 ± 0.7		
≤ 2 people/bedroom	85.2%	---	4.9 ± 2.6
> 2 people/bedroom	14.8%	---	5.9 ± 2.4
Family possessions (number)	9 ± 1.7	5 – 12	
5 -7	24.6%	---	6.1 ± 2.2
8-9	41.0%	---	5.0 ± 3.1
10-12	34.4%	---	4.3 ± 2.1**

[a]Value given as Mean ± SD

**p< 0.05

Exposure characteristics of the participants:

Table 3 represents the potential lead exposure characteristics of the participating children and their families. About 41.3% fathers of the study children were involved in occupations with potential lead exposure (battery recycling, print shop, mechanic, driver, plumber, construction, worker at chemical and paint industry, manufacture of plastic and metals). Mothers who smoked (34.4%) reported smoking on average (SD) 10 (5) cigarettes/ day. Children had higher BLL if the mother smoked ($p< 0.05$) (Table 3). During the summer, about half the study children spent 8 hours or more per week, playing outside their homes. The majority (93.3%) of the households used tap water for cooking and more than half of the families used it for drinking. While 79% of the families reported cleaning the floors more than once per week, less than half (42.6%) the households dusted the house at least once or more than once per week. In most (96.7%) of the households, family members did not take off their shoes when entering the house. Only 31% and 13% families reported using door-mats in all or some of their entrances, respectively. Children's BLL did not differ significantly by other exposure characteristics. The environmental exposure index calculated for each child varied widely with a median (IQR) score of 18 (18). Children's BLL did not differ at the median of environmental exposure index. However, when tertiles of environmental exposure index were created and lower two tertiles were collapsed to create a combined low exposure group (7-23), children in the high exposure group (Environmental index: 28-75) had higher BLL than children in the low exposure group [Mean (SD): 6.0 (3.2) µg/dL vs 4.6 (2.2) µg/dL; $p< 0.05$].

Table 3: Environmental exposure characteristics of 61 children

Characteristics	Median	IQR	%	Blood lead level (µg/dL)[a]
Mother smokes				
No			65.6%	4.5 ± 2.6
Yes			34.4%	6.0 ± 2.5**
Parent's job lead exposure				
No			74.4%	4.9 ± 2.6
Yes			26.6%	5.2 ± 2.7
Child spends playing outside (Hrs/wk)	8	21		
0 – 8			34.4%	4.7 ± 2.1
9 – 25			32.8%	4.5 ± 2.4
26 - 65			32.8%	5.9 ± 3.2
Families using tap water for Drinking				
No			40.0%	4.9 ± 2.7
Yes			60.0%	5.1 ± 2.6
Cooking				
No			6.7%	3.0 ± 0.5
Yes			93.3%	5.2 ± 2.6
Families cleans the floor				
Less than a week			5.0%	5.4 ± 2.9
Once a week			16.4%	5.5 ± 3.6
More than once a week			78.6%	4.9 ± 2.4
Families dust the house				
Less than once per wk			15.0%	5.4 ± 2.9
Once a week			42.6%	5.2 ± 2.6
More than once a week			42.4%	5.2 ± 2.8
Family members take off shoes outside				
No			96.7%	4.9 ± 2.9
Yes			3.3%	5.0 ± 0.7
Families use door-mats				
No			55.7%	5.0 ± 2.8
Yes			31.2%	5.6 ± 2.5
Sometimes			13.1%	3.9 ± 1.6
Environmental exposure index	18	18		
7 – 23			68.9%	4.6 ± 2.2
28 -75			31.1%	6.0 ± 3.2**

[a] Mean ± SD
** $p < 0.05$

Daily dietary intake:

Table 4 presents the mean (SD) and median (IQR) of total dietary calorie and micronutrient intakes of the participating children. The average daily dietary intake of calorie and micronutrients varied widely across the study children (Fig 1-6). Among the 61 children who completed all the evaluation, the average daily calorie intake ranged from 995 to 3956 Kcal. Children with calorie intake more than 3500 Kcal (n=3) were considered as outliers and excluded from further analysis for a final number of 58 children. No difference was detected between included and the excluded children. The median (IQR) daily calorie intake of 58 children was 2188 (695) Kcal (Table 4). There were no significant differences in calorie intake among boys and girls in the study (Table 5). Moreover, calorie intake did not differ by children's weight status (Data not shown). However, daily average calorie intake was significantly correlated with daily average iron ($p< 0.001$), vitamin C ($p< 0.05$) and folate intakes ($p< 0.01$) (Table 4). Calorie-adjusted vitamin C and iron intakes were highly correlated as well (spearman $\rho= 0.3$; $p< 0.05$).

After the calorie adjustment, the median [interquartile range (IQR)] calcium intake of 58 children was 728 (315) mg/day. While half of the children met the Recommended Dietary Allowances (RDA) for calcium established for Uruguayan children (700 mg/day), only 39.7% met the Estimated Average Requirements (EAR) for calcium (800 mg/day) set by the Institute of Medicine (IOM), USA. There were no significant differences in daily calcium intake between boys and girls in the study (Table 5).

Among 58 children, calorie adjusted daily iron intake varied from 5 mg to 52 mg. A child with an iron intake more than 52 mg (Tolerable upper intake levels for iron for this age group: 40 mg/day) was excluded from further analysis. Among 57 children, included in the final analysis, the

median (IQR) daily iron intake was 10 (5) mg/day (Table 4). All children met the EAR for iron (4.1 mg/day) and majority (84%) of the children had intakes greater than or equal to RDA of iron for Uruguayan children (7 mg/day). No significant difference in iron intake was found between the boys and the girls in the study (Table 5). In addition, iron intake was correlated with, serum ferritin level (spearman $\rho = 0.3$; $p < 0.05$).

The median (IQR) daily calorie-adjusted zinc intake for 58 children was 5 (3) mg/day (Table 4). More than half the children had intakes greater than or equal to EAR set for zinc by the IOM. About 32.8% children met the RDA of zinc for Uruguayan children (5.6 mg/day). Boys had more zinc in their diet than the girls [Median (IQR): 5.0 (2.5) mg/day vs 4.0 (2.1) mg/day; $p < 0.05$] (Table 5).

Daily dietary folate intake varied widely among the study children, with a median (IQR) calorie-adjusted intake of 325 (240) µg/day (Table 3). While the majority of the children (81%) met the EAR (160 µg/day) set for US children of similar age group, approximately 45% of the study children had folate intakes lower than the RDA for Uruguayan children (330 µg/day). The girls in the study had more folate intake than boys [Median (IQR): 330 (205) µg/day vs 285 (355) µg/day); however, the difference was not statistically significant (Table 5).

The daily calorie-adjusted vitamin C intake was reported to range from 0-205 mg/day for 58 study children. For biological implausibility, children with reported vitamin C intake of 0 mg/day (n=2) were excluded from further analysis of data concerning vitamin C intakes for a final number of 56 children. Among these 56 children, more than 80% met the EAR for vitamin C (22 mg/day) and approximately 64% of the children met the RDA of vitamin C set for Uruguayan children (35 mg/day). The reported calorie-adjusted median (IQR) vitamin C intake was 44 (37)

mg/day. Although, the girls had more vitamin C intake than boys in the study [Median (IQR): 42 (38) mg/day vs 56 (40) mg/day], the difference did not reach statistical significance (Table 5).

However, daily average intakes of calorie and micronutrients did not vary by socio-economic status or children's weight (data not shown). In ANOVA, we did not find any difference in dietary intake values among the five interviewers. Moreover, no difference in nutrient values was detected among different months of data collection (data not shown).

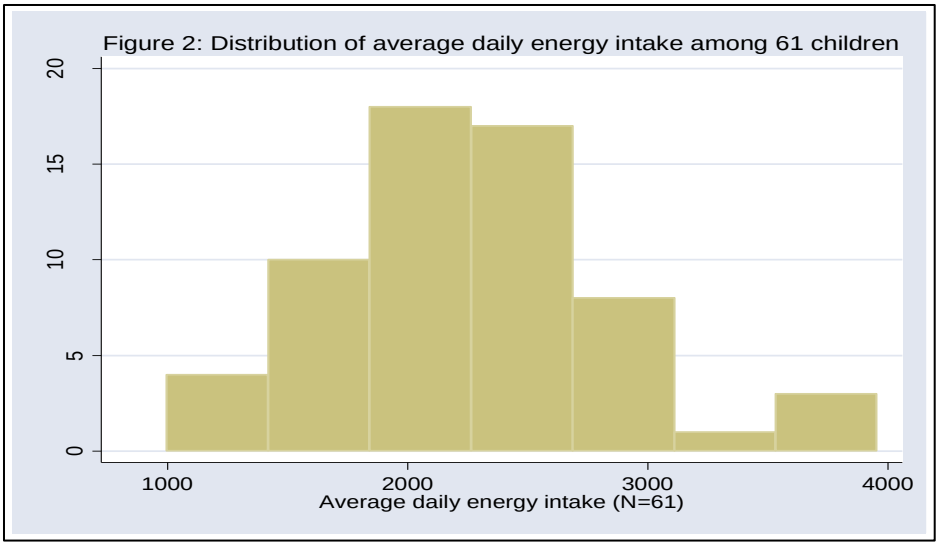
Figure 2: Distribution of average daily energy intake among 61 children
0
5
10
15
20
1000
2000
3000
4000
Average daily energy intake (N=61)

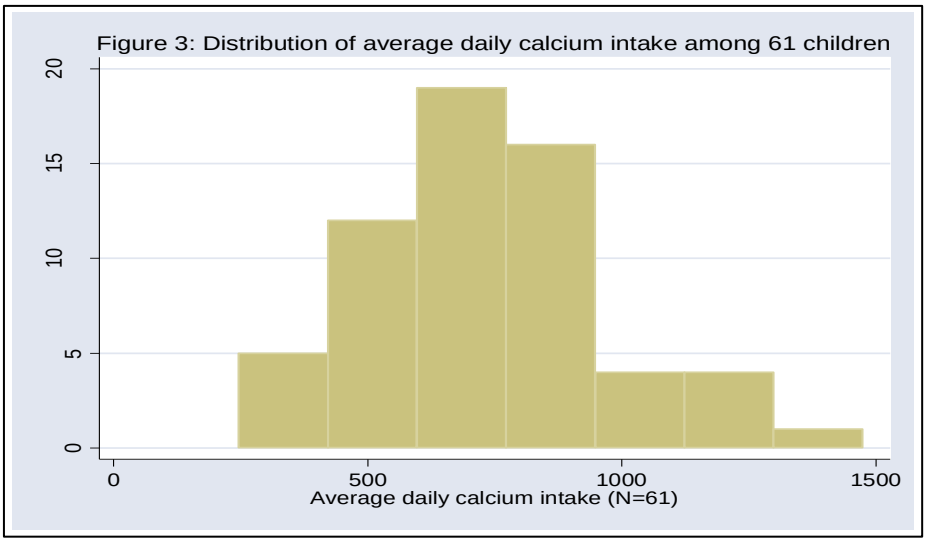
Figure 3: Distribution of average daily calcium intake among 61 children
0
5
10
15
20
0
500
1000
1500
Average daily calcium intake (N=61)

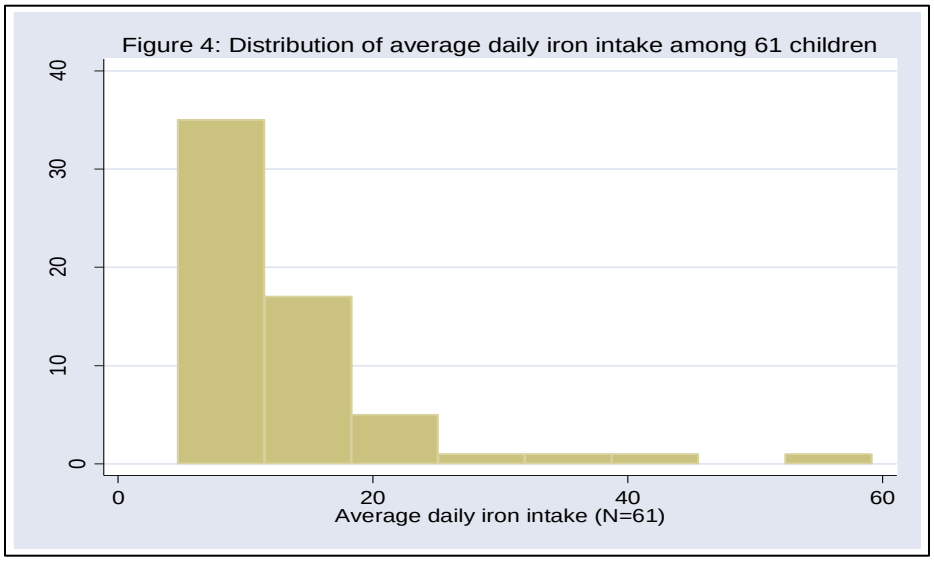
Figure 4: Distribution of average daily iron intake among 61 children
0
10
20
30
40
0
20
40
60
Average daily iron intake (N=61)

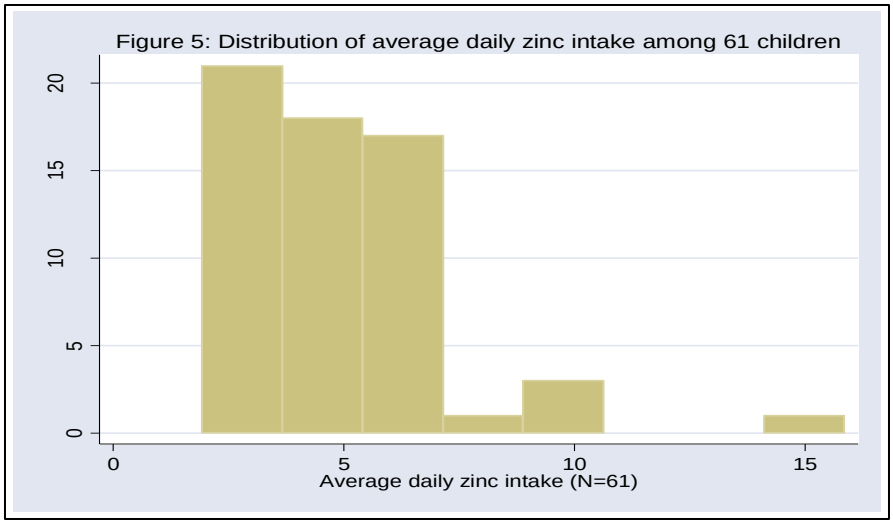
Figure 5: Distribution of average daily zinc intake among 61 children
0
5
10
15
20
0
5
10
15
Average daily zinc intake (N=61)

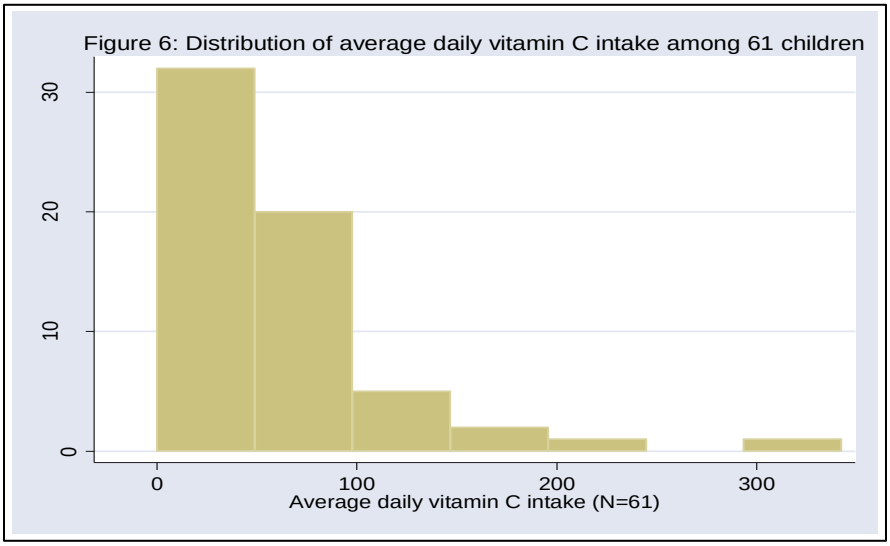
Figure 6: Distribution of average daily vitamin C intake among 61 children
0
10
20
30
0
100
200
300
Average daily vitamin C intake (N=61)

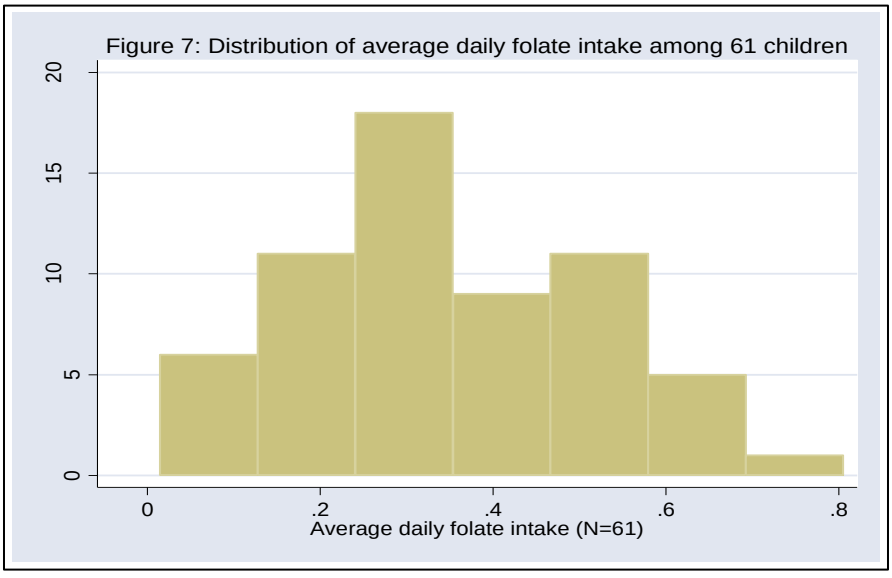
Figure 7: Distribution of average daily folate intake among 61 children
0
5
10
15
20
0
.2
.4
.6
.8
Average daily folate intake (N=61)

Table 4: Daily intakes of nutrients

Nutrient intakes	N	Mean (SD)	Range	Median (IQR)	Children met the requirement (%)
Calorie (Kcal/d)	58	2205 (508)	995 – 3469	2188 (695)	---
Calcium (mg/d)	58	735 (242)	246 – 1474	728 (315)	---
EAR[a] (800 mg/d)		---	---	---	39.7%
RDA[b] (700 mg/d)		---	---	---	56.9
Iron (mg/day)	57	12 (7)	5 – 40	10 (5)	---
EAR[a] (4.1 mg/d)		---	---	---	100%
RDA[b] (7 mg/d)		---	---	---	84%
Zinc (mg/d)	58	5(2)	2 – 14	5 (3)	---
EAR[a] (4 mg/d)		---	---	---	58.4%
RDA[b] (5.6 mg/d)		---	---	---	32.8%
Vitamin C (mg/d)	56	57(43)	6 – 205	44 (37)	---
EAR[a] (22 mg/d)		---	---	---	82%
RDA[b] (35 mg/d)		---	---	---	64.3%
Folate (µg/d)	58	343 (174)	15 – 805	325 (240)	---
EAR[a] (160 µg/d)		---	---	---	81%
RDA[b] (330 µg/d)		---	---	---	44.8%

[a]Estimated average requirements for 4-8 year old US children.
[b]Recommended dietary allowance for 7-10 year old Uruguayan children.

Table 5: Distributions of nutrients by gender

Nutrients	Boys N=36		Girls N=22	
	Mean (SD)	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
Calorie (Kcal/d)	2281(521)	2243 (635)	2081(473)	2121 (633)
Calcium (mg/d)	754 (271)	763 (380)	704 (187)	705 (264)
Zinc (mg/d)	5.5 (2.5)**	5 (2.5)**	4.1 (1.4)	4 (2.1)
Folate (µg/d)	341(149)	330 (205)	347 (211)	285 (355)
	N=36		**N=21**	
Iron (mg/d)	12.4 (7.1)	10 (4.3)	12 (6.9)	10 (5.6)
	N=34		**N=22**	
Vitamin C	52 (36.8)	42 (38)	64 (50.7)	56 (40)

**p< 0.05

Table 6: Correlation between nutrients

	Calories	**Calcium[a]**	**Iron[a]**	**Zinc[a]**	**Folate[a]**
Calories	---				
Calcium[a]	0.2	---			
Iron[a]	0.5***	0.04	---		
Zinc[a]	0.07	-0.02	0.07	---	
Folate[a]	0.4***	0.01	0.2	-0.1	---
Vitamin C[a]	0.3*	-0.2	0.3**	-0.1	0.2

[a]Calorie-adjusted values
***p< 0.01 **p< 0.05 *p< 0.1

Predictors of blood lead concentrations:

The child, family and environmental characteristics that predicted children's BLL in bivariate models were listed in Table 7. Children's BMI was negatively related to BLL ($p < 0.1$). In addition, overweight children had BLL lower by 2.3 µg/dL than the normal weight children ($p < 0.05$), and children who went to the school "Jesus Isaso" had BLL higher by 2.5 µg/dL. Environmental exposure score was directly associated with children's BLL ($p < 0.05$). For each point increase in exposure score, BLL increased by 0.05 µg/dL. A family household possession was inversely associated with children's BLL, with each item increase in household possessions, children's BLL increased by 0.44 µg/dL. Furthermore, family possessions and the school remained most strongly predictive of BLL> 5 µg/dL (Table 7). With one item increase in family possessions, the likelihood of having BLL> 5 µg/dL decreased by 36% (95% CI: 0.44, 0.92) and children from the school "Jesus Isaso" had higher likelihood of having BLL> 5 µg/dL [OR= 9.42, (95% CI: 1.02, 16.0), $p < 0.05$]. However, in bivariate model, serum ferritin or hemoglobin was not a significant predictor of BLL in these children.

Table 7: Predictors of blood lead level and the likelihood of elevated blood lead level (> 5 µg/dL) in 61 study children

	BLL (µg/dL)[a] β [95% CI]	BLL >5 µg/dL[b] OR [95% CI]
Serum ferritin	-0.004[-0.1, 0.1]	
BMI	-0.23 [-0.5, 0.04]*	0.98 [0.79, 1.21]
Overweight[1]	-2.29 [-4.1, -0.51]**	0.36 [0.1, 1.9]
Obesity[1]	-1.04 [-2.7, 0.61]	1.02 [0.28, 3.82]
Family possessions	-0.44 [-0.83, -0.10]**	0.64 [0.44, 0.92]**
School		
Jesus Isaso[2]	2.95 [1.81, 4.01]***	9.42 [1.02, 16.0]***
Environmental exposure	0.05 [0.01, 0.09]**	1.02 [0.99, 1.06]

[a]Predictors tested in simple linear regression and included in the final model.
[b]Predictors tested in logistic regression and included in the final model.
[1]Normal weight used as reference category.
[2]Other schools used as reference category.
*p< 0.1 **p< 0.05 ***p< 0.01

Associations between nutrient intake and blood lead levels:

Calcium intake and blood lead level

Figure 8 represents the distribution of BLL by tertiles of calcium intake. Children who were in the highest tertile of calcium intake (871 - 1474 mg/d) had lower median blood lead level than children who were in the lowest tertile of calcium intake (246 -728 mg/d) [median (IQR): 5.2 (3.1) µg/dL vs 2.9 (2.3) µg/dL; $p< 0.05$].

In regression models adjusted for children's BMI, school, family possessions and environmental exposure index, the association between daily calcium intake and blood lead levels was examined in 58 children. In the covariate-adjusted regression analysis, compared with the lowest tertile of calcium intake (246 – 728 mg/d), children in the highest tertile (871-1474 mg/d) had 1.2 µg/dL lower BLL ($p< 0.1$) (Table 8). Together, the variables in the model explained 41.8% variability in children's BLL and calcium alone explained 10% of the variance of the BLL of the study children. In the logistic regression model, children in the highest tertile of calcium intake had 83% lower risk (95% CI: 0.03, 0.90) of having BLL> 5 µg/dL than children in the lowest tertile ($p< 0.05$) (Table 8).

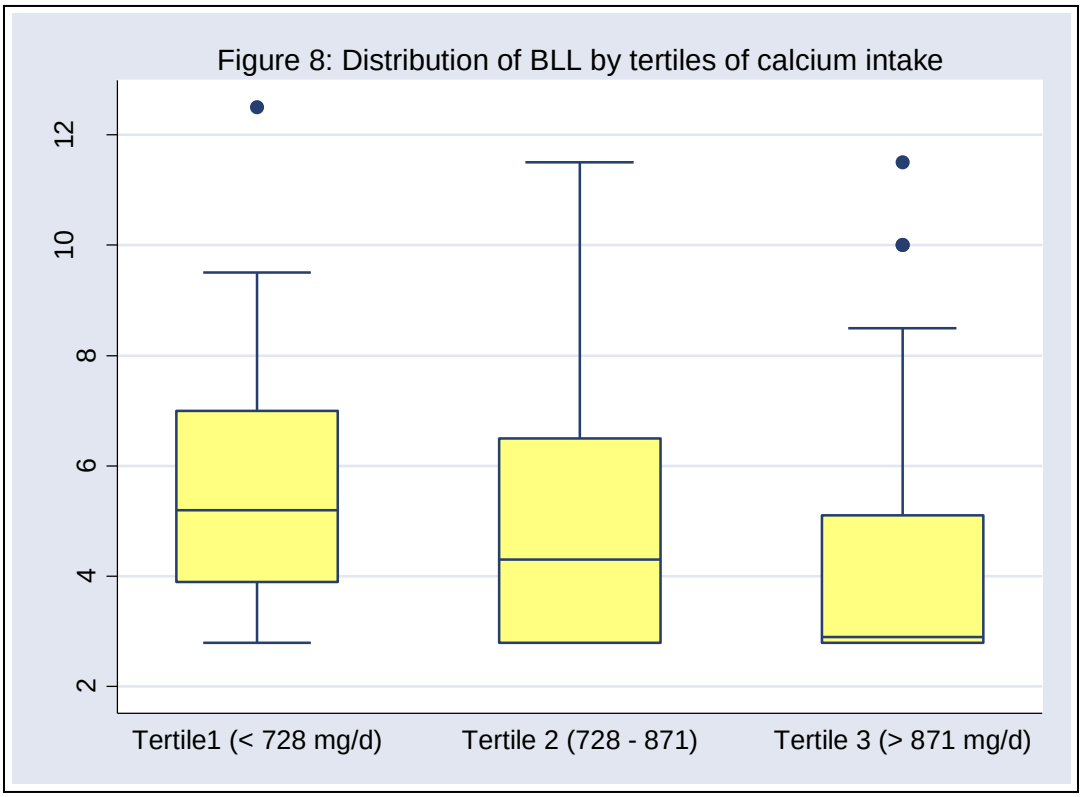
Figure 8: Distribution of BLL by tertiles of calcium intake
2
4
6
8
10
12
Tertile1 (< 728 mg/d)
Tertile 2 (728 - 871)
Tertile 3 (> 871 mg/d)

Table 8: Relation between calcium intake and BLL in 58 study children

Calcium intake[a]	BLL (µg/dL)	BLL >5 µg/dL
	β [95% CI]	OR [95% CI]
Tertile 1 (N=20) (< 728 mg/d)	---[b]	---[b]
Tertile 2 (N=18) (728 - 871 mg/d)	-0.1 [-1.6, 1.3]	0.95 [0.19, 4.89]
Tertile 3 (N=20) (> 871 mg/d)	-1.2 [-2.6, 0.13]*	0.17 [0.03, 0.90]**

[a]adjusted for daily average calorie intake
Regression models adjusted for child's BMI, environmental exposure, family possessions and schools
R^2 of the model= 41.1%
[b]reference group
*p< 0.1 **p< 0.05

Iron intake and blood lead level

Distribution of children's median blood lead levels by iron intake is shown in Figure 9. There was no significant difference in median BLL among children in different tertiles of iron intake.

In multiple regression analysis, after controlling for children's BMI, family possessions, school and environmental exposure scores, there were no significant differences found in BLL and the tertiles of iron intake. Moreover, no significant differences were found in likelihood of getting BLL> 5 µg/dL among children in different tertiles of iron intake (Table 9).

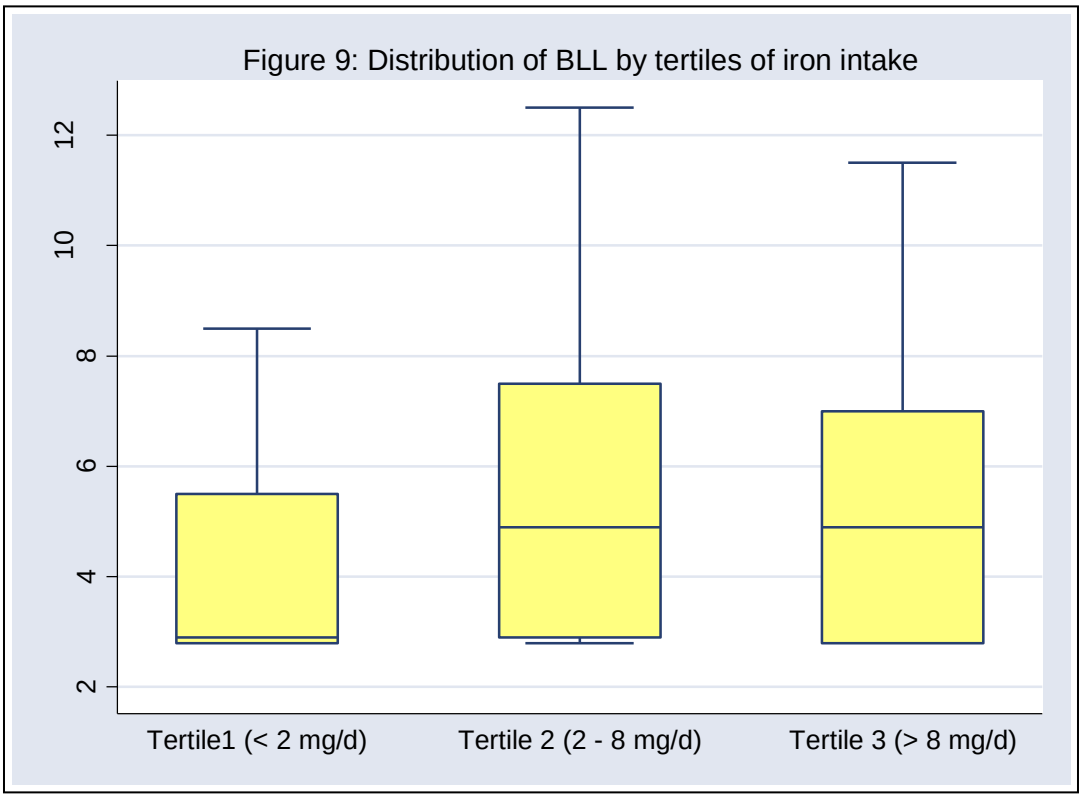
Figure 9: Distribution of BLL by tertiles of iron intake
2
4
6
8
10
12
Tertile1 (< 2 mg/d)
Tertile 2 (2 - 8 mg/d)
Tertile 3 (> 8 mg/d)

Table 9: Relation between iron intake and BLL in 57 study children

Iron intake[1]	BLL (µg/dL) β [95% CI]	BLL >5 µg/dL OR [95% CI]
Tertile 1 (N=19) (< 2 mg/d)	[b] ---	[b] ---
Tertile 2 (N=19) (2-8 mg/d)	-0.86 [-2.4, 0.65]	0.49 [0.07, 3.38]
Tertile 3 (N=19) (> 8 mg/d)	-0.70 [-2.2, 0.77]	0.48 [0.06, 3.76]

ion

Zinc intake and blood lead level

Distribution of BLL among the tertiles of zinc intake is presented in Figure 10. The median BLL did not significantly differ across the tertiles of zinc intake in 58 study children.

When tested in multiple regression models, after controlling for children's BMI, family possessions, school and environmental exposure scores, there was no significant difference in BLL across the tertiles of zinc intake. Moreover, in logistic regression analyses, the risk of having BLL> 5 µg/dL, did not differ across the children in different tertiles of zinc intake (Table 10).

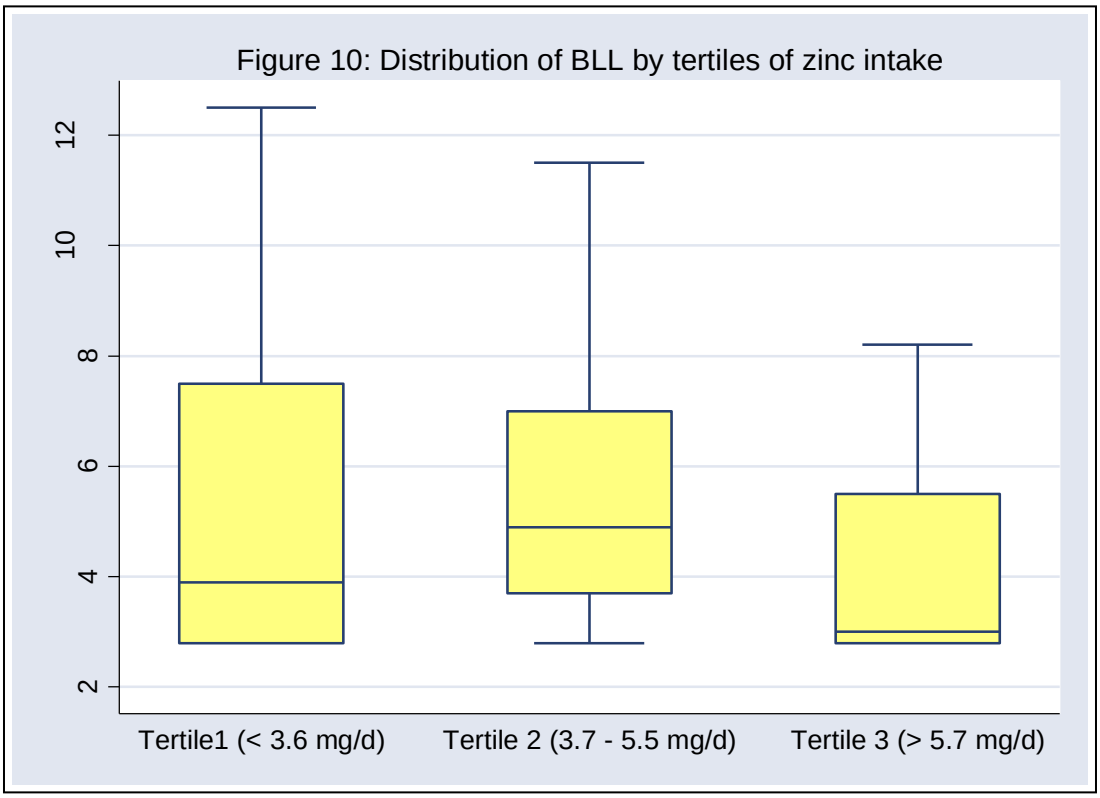
Figure 10: Distribution of BLL by tertiles of zinc intake
2
4
6
8
10
12
Tertile1 (< 3.6 mg/d)
Tertile 2 (3.7 - 5.5 mg/d)
Tertile 3 (> 5.7 mg/d)

Table 10: Relation between zinc intake and BLL in 58 study children

Zinc intake[a]	BLL (µg/dL) β [95% CI]	BLL >5 µg/dL OR [95% CI]
Tertile 1 (N=20) (< 3.6 mg/d)	[b] ---	[b] ---
Tertile 2 (N=19) (3.7 – 5.5 mg/d)	-0.02 [-1.4, 1.3]	0.56 [0.12, 2.68]
Tertile 3 (N=19) (> 5.7 mg/d)	-0.64 [-2.0, 0.72]	1.6 [0.15, 3.52]

[a]adjusted for daily average calorie intake
Regression models adjusted for child's BMI, environmental exposure, family possessions and schools
R^2 of the model= 52.0%
[b]reference group

Vitamin C intake and BLL

Figure 11 represents the distribution of BLL across the tertiles of vitamin C intake in 56 children. Surprisingly, children in the highest vitamin C intake group had higher median BLL than children in the lowest vitamin C intake group [median (IQR): 5.3 (4.2) mg/day vs 4 (2.6) mg/day; $p< 0.1$].

In multiple regression analysis, adjusted for children's BMI, school, family possessions and environmental exposure, there was no significant difference in BLL across the tertile of vitamin C. In logistic regression model, no significant difference was observed in likelihood of having BLL> 5 µg/dL among children in different tertiles of vitamin C intake (Table 11).

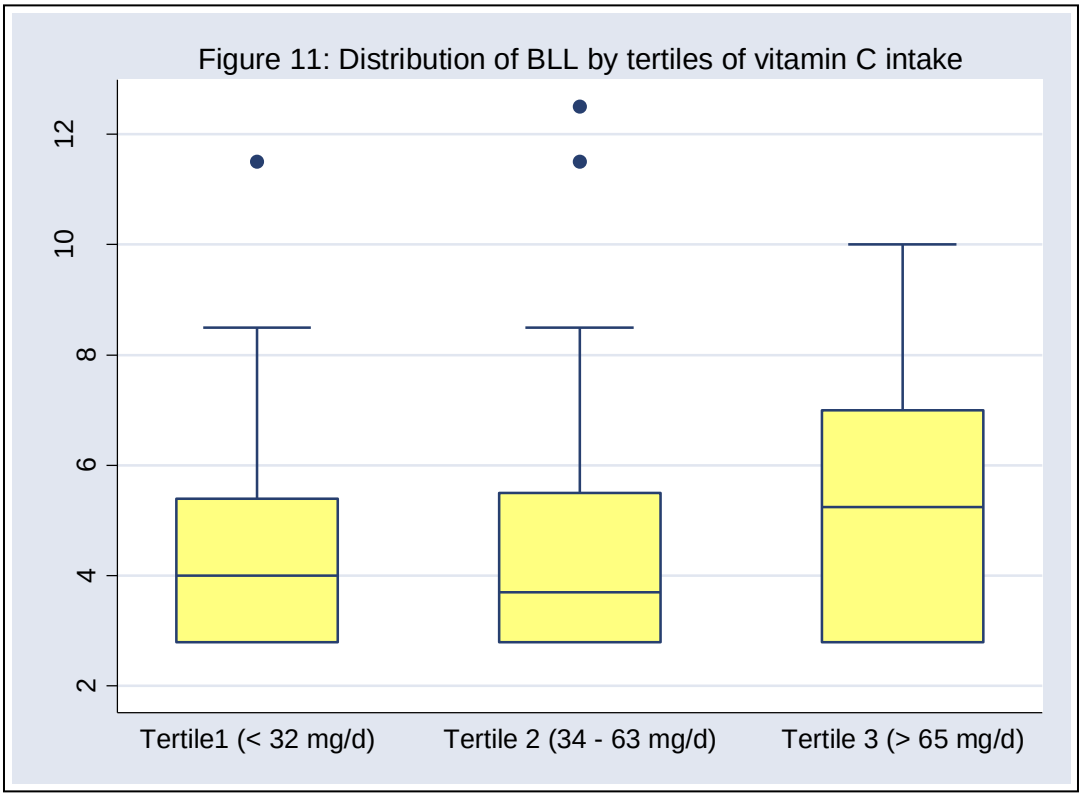
Figure 11: Distribution of BLL by tertiles of vitamin C intake
2
4
6
8
10
12
Tertile1 (< 32 mg/d)
Tertile 2 (34 - 63 mg/d)
Tertile 3 (> 65 mg/d)

Table 11: Relation between vitamin C intake and BLL in 56 study children

Vitamin C intake[a]	BLL (µg/dL) β [95% CI]	BLL >5 µg/dL OR [95% CI]
Tertile 1 (N=19) (< 32 mg/d)	[b] ---	[b] ---
Tertile 2 (N=19) (34-63 mg/d)	0.41 [- 1.03, 1.84]	2.57 [0.33, 20.0]
Tertile 3 (N=18) (> 65 mg/d)	0.42 [-1.01, 1.86]	3.52 [0.45, 27.57]

[a]adjusted for daily average calorie intake
Regression models adjusted for child's BMI, environmental exposure, family possessions and schools
R^2 of the model= 51.3%
[b]reference group

Folate intake and BLL

Distribution of children's median BLL across the tertiles of folate intake was shown in Figure 12. Children's median BLL did not differ significantly across the tertiles of folate intake.

In the multiple regression models, no significant difference was observed among children in different tertiles of folate intake. There was also no significant difference in risk of having a BLL> 5 µg/dL among the children in different tertiles of folate intake (Table 12).

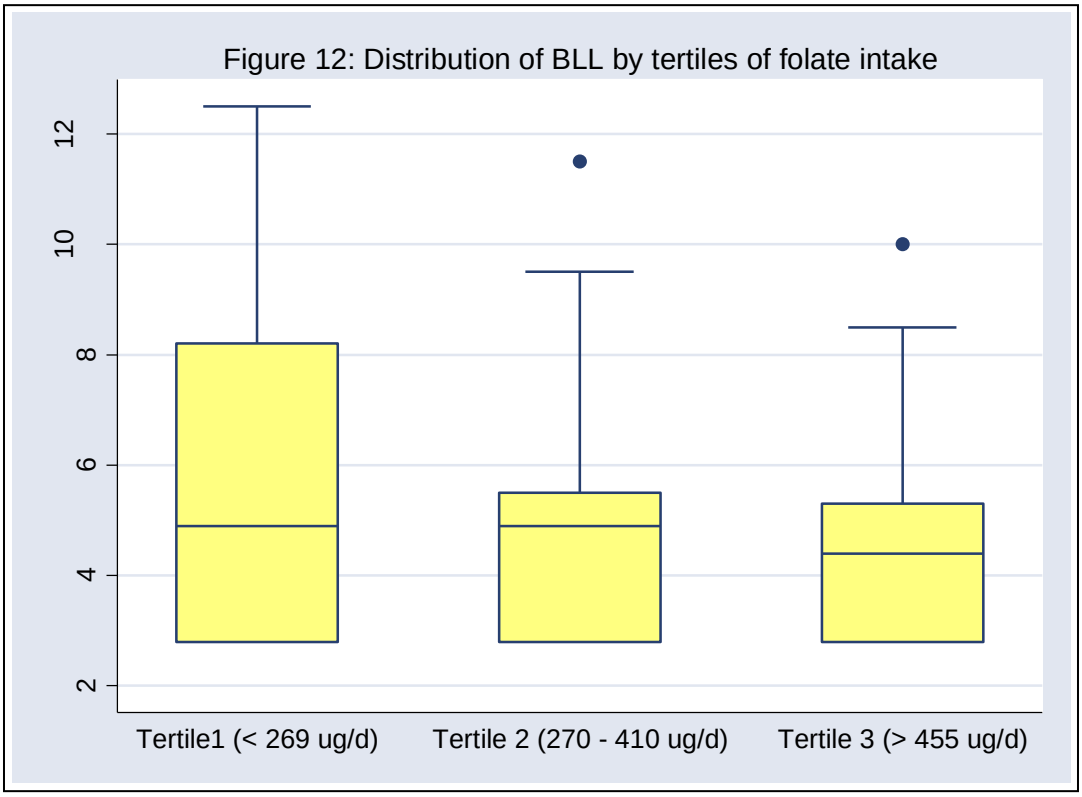
Figure 12: Distribution of BLL by tertiles of folate intake
2
4
6
8
10
12
Tertile1 (< 269 ug/d)
Tertile 2 (270 - 410 ug/d)
Tertile 3 (> 455 ug/d)

Table 12: Relation between folate intake and BLL

Folate intake[1]	BLL (µg/dL) β [95% CI]	BLL >5 µg/dL OR [95% CI]
Tertile 1 (N=21) (< 269 µg/d)	---[b]	---[b]
Tertile 2 (N=18) (270 - 410 µg/d)	0.58 [-0.73, 1.89]	2.28 [0.4, 29.6]
Tertile 3 (N=19) (> 455 µg/d)	-0.76 [-2.10, 0.54]	0.37 [0.07, 1.91]

[a]adjusted for daily average calorie intake
Regression models adjusted for child's BMI, environmental exposure, family possessions and schools
R^2 of the model= 52%
[b]reference group

Effects of nutrient interactions on BLL

When the interaction terms between tertiles of calcium and iron intakes were included in the linear regression models, we did not find any significant effect of the interaction between calcium-iron and iron-vitamin C on children's BLL (data not shown).

Chapter 4

DISCUSSION

In this sample of 6-8 year old school children from Montevideo, Uruguay, calcium intake was inversely associated with BLL. Children in the highest tertile of calcium intake had significantly lower BLL than children in the lowest tertile. This result is consistent with results found in other studies (Schell et al 2004; Hertz et al 2000; Mahaffey et al 1986; Blake and Mann 1983; Heard and Chamberlain 1982; Johnson and Tenuta 1979; Sorrell et al 1977; Mahaffey et al 1973). However, dietary intakes of iron, zinc, folic acid and vitamin C were not significantly associated with the BLL in the study children. This is one of the first studies to examine the relation between dietary intake of multiple micronutrients and blood lead levels in school children.

Blood lead levels of the study children:

The mean BLL of the study children were lower than the BLL found in preschool children from Montevideo (Queirolo et al 2010). This finding is not surprising since children's BLL typically peak during the first 3 years of life, before gradually declining (Dietrich et al 2001; McMichael et al 1985). Moreover, this study was conducted one year after the study in preschoolers and a lower trend in BLL is expected in studies conducted further away in time from the year lead was removed from the gasoline. However, with a mean (SD) of 5.0 (2.6) µg/dL, about 8.2% children having BLL> 10 µg/dL and 37.7% children with BLL> 5 µg/dL, the extent of lead exposure of the study children was comparable with the children of the similar age group from other Latin American countries. For example, in 5-9 years old children from

Cartagena, Columbia, the mean (SD) BLL was 5.5 (0.23) µg/dL, with 7.4% of the children having BLL≥ 10 µg/dL and 11.6% with BLL> 5 µg/dL (Olivero-Verbel et al 2007). In 4-16 year old children from the city of Zarate, Argentina, the mean (SD) BLL was 6.3 (6.0) µg/dL, with 14.4% of the children having BLL≥ 10 µg/dL (Beltramino et al. 2007). Moreover, the mean (SD) BLL of 213 children of similar age group (5-16 year old) from Posada, Argentina was found to be 5.8 (4.3) µg/dL, with 16% children having BLL≥ 10 µg/dL (Beltramino et al. 2007). On the other hand, some studies in Latin America reported higher BLL in children than the study children. For example, in the State of Morales, Mexico, children 1 to 12 years of age had mean (SD) BLL of 8.2 (5.6) µg/dL, with 29.7% having BLL≥ 10 µg/dL (Meneses-González et al. 2003). However, the BLLs in the study children were higher than in US children of similar age who experience population average BLL of approximately 1.1 µg/dL (CDC, 2005). With detrimental effects of lead exposure on IQ and behavior found even at a BLL < 5 µg/dL, this puts a significant number of the study children at risk of potentially irreversible cognitive deficits (Canfield et al 2004).

Factors affecting children's blood lead levels:

Children's BMI was a significant predictor of their BLL in this study. In bivariate model, with 1kg/m^2 increase in BMI children's BLL decreased by 0.23 µg/dL ($p< 0.1$). We don't know the exact reason of this relationship, but it is possible that higher BMI is signifying higher socio-economic status in these children. It is also possible that children with higher BMI had higher calorie and protein intake and higher calorie and protein intake is associated with lower BLL in some studies (Lucas et al 1996; Lanphear et al 2002). On the other hand, family household possession was significantly associated with children's BLL and possessions of more household items were related to lower likelihood of having BLL> 5 µg/dL. Studies consistently found an

inverse association between socio-economic status and children's BLL (Carrillo et al 1996; Romeiu et al 1992; Mathee et al 2006). This is not surprising, since parents of children from families with higher resources may be less likely to be involved in activities/jobs that expose children to lead or live in areas with less industry. Moreover, children from low-income families may be more likely to live in older houses with lead based paint or in housing conditions that make them more vulnerable to lead exposure. Further, family's income is also related to household food-security and nutritional status. Children from low-income families are more at risk of poor nutrition (Casey et al 2001; Caballero, 2005). Interestingly, school was a significant predictor in the study. Children from the school "Jesus Isaso" had higher BLL and higher risk of having BLL> 5 µg/dL. This may be due to the location and the socio-economic status of the children attending the school. This particular school is located in an area surrounded by garment and paint industries which may be a potential source of exposure to lead. Moreover, children of this school came from lower socio-economic status (fewer household items) than children of other participating schools. As expected, higher environmental exposure index was a significant predictor of higher BLL in the study children.

Daily dietary intake:

There was a significant variability in calorie consumption among the study children, ranging from 995 to 3956 Kcal/day. We did not include children with calorie intake more than 3500 Kcal/day in the final analysis. We decided to use this cut-off since the 95^{th} percentile for calorie intake among our study children was 3469 Kcal/day. Moreover, the 95^{th} percentile for daily calorie intake among children aged 5 to 8 years in NHANES III was 2463 Kcal/day (CDC 2003). The mean calorie intake of 58 children was comparable to that of US children of similar age group (CDC 2003). According to data from NHANES III, 6-11 year old US children

consumed on average 2025 Kcal/day, whereas our study children had a calorie intake of 2205 Kcal/day. Although modest prevalence of overweight (16%) and obesity (20%) was observed in these children, daily calorie intake was not significantly associated with children's weight status.

Daily calcium intake

With a median calcium intake of 728 mg/day and about 43% of the study children having intakes below the RDA for calcium for Uruguayan children of the same age group, intake of calcium in our study children is comparable to intakes found in children from Chile and Argentina (Leiva et al 1995; Abeyá et al 2009). In 10-14 year old school children from Santiago, Chile, the median intake of calcium were 735 mg/day for boys and 648 mg/day for girls, with 40% of the children less than 11 years of age having intakes below the RDA (Leiva et al 1995). In a National Health and Nutrition Survey conducted in 2004-'05, the median calcium intake of 2-5 year old children was 700 mg/day and about 46% of the children did not meet the recommendation for calcium for this age group (Abeyá et al 2009). On the other hand, children in our study had lower average daily calcium intake than US children of similar age group (CDC 2003). While 6-11 years old US children who participated in NHANES III had a daily intake of 889 mg of calcium per day, our study children had a mean calcium intake of only 735 mg/day. Majority of our study children (60%) had calcium intake below the EAR for calcium for US children. In absence of the data on frequency of food consumptions, it is difficult to comment on the low calcium intake in these children, but it is possible that calcium intake is low in these children due to lower intakes of dairy products. This explanation fits with the anecdotal evidence from the study's pediatrician who believes that dairy consumption is indeed low in Uruguayan population, replaced by the very popular beverage, mate.

Iron intake

In contrast to calcium intake, iron intake in this sample of school children was similar to US children of similar age group. The average daily calorie-adjusted iron intake of our study children was 12 mg, whereas 6-11 year old US children had a mean iron intake of 14.4 mg/day (CDC 2003). Similarly, iron intake of our study children was comparable to children from Argentina. In 6-12 year old children from Rosario, province of Santa Fe, Argentina, the mean intake of iron in boys and girls were 14.7 mg/day and 12.9 mg/day, respectively (Bonzi & Luna, 2008). All of our study children met the EAR for iron and almost 84% of the children met the RDA for iron for Uruguayan children. Anemia was not prevalent, but majority (77.5%) of the children was iron deficient. However, at the time of analysis of these results, data on biomarker of infection/inflammation was not available. Since, ferritin is an acute-phase protein, normal or elevated levels can be observed in presence of parasitic infection and inflammation (Tomkins 2003). Serum ferritin was positively correlated with iron intake in this study. Although, statistically not significant, but children with iron deficiency had lower iron intake than children without iron deficiency. One child who was identified as anemic had daily iron intake of 4.7 mg/day. For the current study, we did not have information on amount of meat intake by these children. However, since Uruguay is a country with the highest per capita meat consumption in the world (Speedy et al 2003), the high intake of iron observed in these children may be due to high consumption of meat and meat products.

Zinc intake

On the other hand, mean daily zinc intake of the study children was lower than the average zinc intake in 6-11 year old US children. While children in our study had a mean zinc intake of 5 mg/day, US children of similar age group had a mean intake of 10.6 mg zinc/day

(CDC 2003). However, more than half of the study children met the EAR (4 mg/day) and almost 33% met the RDA for zinc for Uruguayan children (5.6 mg/day). Lack of studies from Uruguay or from the neighboring countries has made it difficult to compare the intakes of our study children with intakes found in other studies from the region. However, in a randomized clinical trial with zinc in Santiago, Chile, at baseline, 6-12 year old children had a mean intake of 6.5 mg zinc/day, with about 50% children having intake below the RDA for zinc for (Castillo-Durán et al 1995). Boys had higher mean zinc intake than girls in our study. Sex difference in dietary zinc intake has been seen in other studies. In a nationally representative data from NHANES III, Briefel et al (2000) observed significantly higher intake of zinc in adolescent and adult males than in females of similar age. Higher calorie intake in males than females may be one of the reasons for the difference in zinc intake among sexes (Briefel et al 2000). In our study, the boys had higher calorie intake than girls, but the difference was not statistically significant. It is not clear why gender difference in zinc intake was observed at such a young age. Although, consumption of meat, a good source of zinc, is high in Urguayan population, other foods rich in zinc (e.g. oyesters, shellfish) is not common in regular Uruguayan diet. We do not have information on frequency and types of food consumption in these children, but a vegetarian diet or a diet predominantly containing unfortified cereals and/or fruits and vegetables may have contributed to zinc intake below the RDA for about 67% of the study children. Fortification of cereals with vitamins and minerals is not mandatory in Uruguay and most cereals including breads, one of the frequently consumed foods in this population, are not fortified with zinc. A substantial proportion of study children not meeting the RDA for zinc pose concerns, since this may have adverse effects on their growth and development, cognitive functions and immune systems (Hambridge 2000).

Vitamin C and folate intake

Majority of the study children had adequate vitamin C and folate intakes. However, compared to 5 to 8 year old US children who participated in NHANES III, children in our study had lower intake of vitamin C (CDC 2003). On the other hand, daily dietary folate intake of the study children was similar to that of the US children of similar age group (CDC 2003). In US, foods fortified with folate (cereals, milk) are a good source of this nutrient. However, in Uruguay, foods are not fortified with folate. In absence of data on frequency of fruits and vegetable consumptions, it is difficult to comment how the majority of the study children met their vitamin C and folate intakes. Moreover, intake of small number of children of our study may not be reflective of vitamin C and folate intake of Uruguayan children. Furthermore, intakes of these micronutrients may vary widely between days and seasons and two 24 hour dietary recalls may not be adequate to find out the actual intake of these vitamins. However, dietary intake data in this study did not differ by study months. Over-reporting of fruits and vegetable intake may also be possible if viewed as socially desirable or healthy. However, probing questions were asked to verify accuracy of the answers. Finally, data on plasma vitamin C and serum folate may confirm the accuracy of this dietary folate data.

Calcium intake and blood lead level:

Daily calcium intake was inversely associated with BLL in the study children. This is consistent with the results found in studies in both children and adults (Mahaffey et al 1986; Hertz et al 2000; Schell et al 2004; Black and Mann 1983; Heard and Chamberlain 1982; Johnson and Tenuta 1979; Mahaffey et al 1973; Sorrell et al 1977). For example, Schell et al (2004) found significant inverse relationships of BLL of infants at 6 months of age with their calcium intake. Dietary intake in that study was assessed by 24 hour recalls at 3 monthly

intervals. However, at 12 months of age calcium intake was no longer associated with blood lead level in these children. The authors explained the shift as a result of a greater percentage of the study children becoming calcium deficient at 12 month of age and the difference in calcium intake was insufficient across the physiological range of activity to produce the variation in outcome of BLL. Moreover, the BLL increased at 12 months and increasing exposure of older infants overwhelmed the effects of dietary influences (Schell et al 2004). Mahaffey et al (1986), on the other hand, showed that dietary calcium intake measured by a 24 hour dietary recall was inversely associated with blood lead level in 1-11 year olds with a mean BPb of 15.7 µg/dL. Results of their study showed that children's calcium intake had a small, inverse correlation with their BLL, with children's BLLs declining by about 0.2% for each 100 mg increase in dietary calcium (Mahaffey et al 1986). In adults, higher calcium intake has been associated with lower BLL in pregnant and lactating women (Hertz et al 2000, Ettinger et al 2006). For example, in a cohort of 195 US women who were followed through their pregnancy, higher calcium intake was associated with lower BLL (Hertz et al 2000). With a mean BLL< 5 µg/dL, the extent of current lead exposure was low in those pregnant women and some benefit of calcium intake even above the RDA was observed during the latter half of pregnancy. Moreover, an inverse association was found between high milk intake and BLL among 98 lactating women from Mexico City (Hernández et al 2003). Although, results of a large number of studies showed protective effect of calcium intake on BLL, there was still inconsistency in results on the level of lead exposure at which calcium has the most beneficial effect. Our results have provided evidence that higher calcium intake is related to lower BLL even at low level of exposure.

In contrast to our results, Lanphear et al (2002) did not find any significant association between calcium intake and infant's BLL when infants were followed from 6 to 24 months. But,

on average, children in their study had very high calcium intakes at every stage of infancy. Moreover, infancy may not be an appropriate age for examining the relation between calcium intake and BLL since at that young age children's diet normally consists of fairly high amounts of dairy products. In contrast, One notable result in our study was that children in the highest tertile of calcium intake (>871 mg/day) had 1.2 µg/dL lower BLL than children in the lowest tertile of calcium intake (< 630 mg/day). Moreover, children in the highest tertile of calcium intake had lower likelihood of having a BLL> 5 µg/dL. The EAR for calcium for this age group is 800 mg/day. It is possible that children who did not meet the EAR for calcium were more vulnerable of having a higher BLL than those who met the requirement. The possible mechanism behind this inverse association between calcium intake and BLL may be the decreased intestinal absorption of lead in the presence of adequate calcium since calcium and lead compete for similar binding sites on intestinal mucosal proteins (Barton et al 1978).

Iron, zinc intake and blood lead level:

We did not find any association between iron or zinc intake and children's BLL. Almost all the children in our study had adequate intake of iron for their age group and anemia was also not a problem in these children. Although, serum ferritin levels were low in these children, no association was found between ferritin levels and BLL in 61 study children. Majority of the study children also had adequate zinc intake for this age group. In contrast to our findings, some studies found an inverse association been dietary iron or zinc intake and BLL in young children (Lanphear et al 2002, Hammad et al 1996, Schell et al 2004). In a longitudinal analysis, children who were followed from 6 months to 2 years of age, Lanphear et al (2002) found a significant inverse relationship between iron intake and BLL during the first year of life. A high proportion of the children were iron insufficient in that study. However, at 18 months and 24 months of age

the association between iron intake and children's BLL was no longer significant. During the first year of life, infants are vulnerable to low iron intake if adequate iron is not provided by the complementary foods since breast milk provides a small amount of highly bioavailable iron (Duggan et al 2008). But as they grow older, more foods are introduced in infant diet and the possibility of getting adequate iron from the diet increases. Thus in many studies the inverse relation between iron intake and BLL has disappeared at older age.

In 299 urban preschool children aged 9 to 60 months, Hammad et al (1996), found an inverse relation between iron intake and BLL, with only the children in highest quartile of iron intake having a lower level of lead. The authors did not mention the amount of iron intake among the highest quartile of children. Therefore, it is difficult to understand at what intake level iron may have a beneficial effect on BLL. Moreover, the mean BLL of children in that study was quite higher than in our study [Mean (SD) BLL: 11.4 (7.3) µg/dL]. Schell et al (2004) observed significant inverse relationships between BLL and 6 month old infants' intakes of iron and zinc. At 12 months, low iron intake continued to be associated with higher lead levels in these young children, although zinc did not. Notably, in this study, proportion of children not meeting the RDA for iron increased from 1.2% to 20.1% from 6 months to 12 months of age, but for zinc intake, the percentage of children not meeting the RDA for zinc intake decreased from 37.9% to 26.1% (Schell et al 2004).

There may be several reasons behind null association between iron or zinc intake and BLL in our study. First, the sample size of our study may be too small to detect any difference in BLL among different tertiles of iron or zinc intake. Second, the iron and zinc intake of majority of our study children were adequate. With adequate intake, there may not be additional benefit of these micronutrients on children's BLL. Furthermore, two 24- hour dietary recalls may not be

sufficient to assess the daily variation of iron and zinc intake in these children. However, serum ferritin levels were low in these children and iron intake was correlated with ferritin levels. It is possible that serum ferritin levels were low due to insufficient iron absorption as a result of inadequate vitamin C intake or inhibitory effects of other dietary factors. Although, vitamin C intake in majority of the study children were adequate, confirmation is only possible with availability of data on plasma levels of vitamin C of these children. We don't have information on dietary factors that may inhibit iron absorption in these children. The difference between previous studies and ours is that we calculated the calorie-adjusted values of the nutrients by residual analysis, whereas many studies (Hammad et al 1996; Lanphear et al 2002; Schell et al 2004) have included total calorie intake as a co-variate in their multiple regression models which may not provide a calorie independent absolute values for nutrients that can be achieved by residual analysis.

Vitamin C, folate intake and blood lead level:

We did not find any association between vitamin C or folate intake and BLL. Although, several studies (Schell et al 2004; Hertz et al 1998; Cheng et al 1998; Simon et al 1998) have measured dietary vitamin C intake and examine its association with BLL in both adults and children, but only few studies (Cheng et al 1998; Schwartz et al 1989) found an inverse association between vitamin C intake and BLL. In a cross-sectional examination of 49-93 year old men in Normative Aging Study, after controlling for patella lead, age, smoking, and alcohol consumption, men in the lowest vitamin C intake quintile (< 109 mg/day) had a mean blood lead level 1.7 μg/dL higher than men in the highest quintile (≥ 339 mg/day). A strong inverse relation between vitamin C intake and blood lead level was also reported in a study of 9,996 subjects in the Second National Health and Nutrition Examination Survey (Schwartz et al 1989). On the

other hand, although Simon and Hoods (1998) found an inverse association between serum vitamin C and BLL in both children (6-16 years old) and adults from NHANES III, there was no association between dietary intake of vitamin C and BLL in their study. The authors argued that the discrepancy in the result may be due to the impreciseness of the dietary estimates or due to the effects of elevated blood lead levels, increasing the turnover of vitamin C, thereby lowering serum ascorbic acid levels (Simon and Hoods, 1998). We do not have plasma vitamin C data for our study children to test whether the latter hypothesis is true and whether there is an association between plasma vitamin C and BLL in these children. Physiologically, plasma vitamin C concentrations are maintained between 10 -160 μM and any excessive intake is excreted by the kidney (Levine et al 1996). Therefore, higher intake of vitamin C may not be biologically available to exert its effect. Since majority of our study children had adequate intake of vitamin C, it may have limited our ability to detect any beneficial effect of vitamin C on BLL. In contrast, our study found a non-significant but positive association between vitamin C intake and children's BLL. The physiologically plausible explanation for this relationship may be the fact that a high proportion of our study children had iron deficiency and since the expression of the intestinal divalent metal transporter protein DMT1 increases with iron deficiency and since lead competes for the similar binding site on DMT1 for absorption, in presence of iron deficiency lead absorption may have increased in these children.

Epidemiological research with dietary folate intake and lead exposure is very scarce. In fact ours is one of the first studies examining the relationship between folate intake and BLL in children. However, in US women of reproductive age, Lee et al (2008) observed a positive association between dietary folate intake and BLL and an inverse relationship between serum folate and BLLs. The authors suggested that folate in blood may enhance excretion of lead, while

dietary folate may facilitate lead absorption. In animals, the supplementation of folic acid resulted in mobilization of tissue lead and enhanced urinary excretion of lead (Tandon et al. 1987). However, the mechanism by which folate interacts with lead is not well understood. We cannot comment on the serum folate level, but in our study, most of the children had adequate intakes of folate in their diet. Lack of power may be one of the reasons for our failure to detect any significant association between vitamin C or folate and children's BLL.

Study Strengths:

There are several strengths of our study. To our knowledge, ours is one of the first studies looking at the dietary intakes of micronutrients and blood lead levels of 6 to 8 year old children. Although, there have been studies examining the relation between nutritional factors and blood lead level in infants, pregnant and lactating women, there was a lack of studies focusing on school age children. This is an important age group to study. Because, first, older children's dietary habits and nutritional needs are different from infants or pregnant and lactating women and thus the results from previous studies cannot be extended to school children. Second, finding an effective preventive strategy against elevated blood lead level at this age will not only help to decrease children's blood lead level but in turn will help preventing lead induced cognitive and behavioral deficits that can have important implications in later life.

We have identified several socio-demographic and environmental factors that can influence BLL in these children and controlled for these explanatory variables in our final regression model examining the intake of each nutrient and BLL. Many previous studies (Schell et al 2004, Hammad et al 2002), did not account for environmental lead exposure, which might have influenced the estimation of the effects. Moreover, unlike other studies, where total daily calorie intake was included as a covariate in the regression models, we have calculated calorie-

adjusted nutrient intake. This technique is statistically more robust since it provides calorie independent values of nutrients by adjusting individual variation of calorie intake.

Many of the previous studies on nutritional intake and BLL were conducted in US population. There is a need to provide information from other cultures that may exhibit unique patterns of exposure. Moreover, country/region specific differences in dietary habits may provide a unique opportunity to re-examine the findings of the previous studies. Finally, it is important to identify a population at risk for both micronutrient/s deficiency and lead exposure to help formulating intervention strategies to reduce exposure and eliminate deficiencies. Our study has provided evidence for the presence of both inadequate calcium intake and lead exposure in these Uruguayan school children. Calcium supplementation along with strategies to increase calcium intake may lower BLL and improve calcium status of these children.

Study limitations:

As in all studies, there are limitations of the current investigation. First limitation to consider relates to accuracy and effectiveness of the diet assessment tool in capturing the daily diet of the children. Two 24 hour dietary recalls may not be adequate for collecting dietary information in children of this age group. However, there was little variability observed in the daily diet of these children. As with other diet assessment methods, dietary recalls are subject to memory and respondent bias which may have influenced our findings. However, we facilitated recall by the mother or the caretaker and the child by asking open-ended questions.

Lack of data on frequency of specific food intake has made it difficult for us to comment on the dietary pattern of the study children. In addition, data on biomarkers for many of these nutrients were not available at the time of this analysis, thus validation of the dietary data could not be done. Moreover, the nutrient data base for Uruguayan foods that was used in this study to

calculate nutrients in children's diet has not been validated. Nevertheless, this particular nutrient database is widely used to determine nutrient intakes of the Uruguayan population.

Another important limitation of our study is a potential lack of power. A sample size of 61 children may not be adequate to find any statistically significant associations between nutrient intakes and blood lead level. However, as an exploratory study, the findings generated here allow us to formulate hypothesis to be tested with larger sample sizes in the future.

Finally, one limitation of this study is its cross-sectional design because it precludes us from establishing causality. Better understanding of the effects of nutrients on BLL could be obtained by conducting a well-designed randomized clinical trial with calcium and other micronutrients. However, to design such a trial, a clear understanding of exposure levels, dietary intakes and the associations between BLLs and nutrients is fundamental.

Chapter 5

CONCLUSIONS

In summary, the results from the present study suggest that higher calcium intake is associated with lower blood lead level in children. However, we did not find any association between intakes of iron, zinc, vitamin C and folate with children's blood lead level. The majority of our study children had adequate intakes for other micronutrients. However, our study is limited by a small sample size and lack of data on environmental exposure such as water, soil and dust lead levels. Future research should include these parameters into their studies to better understand the dynamics of environmental lead exposure, blood lead level and nutritional factors which ultimately will help to develop better preventive measures against childhood lead toxicity.

This study provides additional evidence to the findings of our previous study (Queirolo et al 2010) and studies by others (Mañay et al 2008; Cousillas et al. 2005) that pediatric lead exposure continues to be a problem in Uruguayan children despite the elimination of leaded gasoline in 2004. Although the extent of lead exposure in these children was lower than in Uruguayan pre-schoolers, with the growing body of literature showing harmful effects of low level of lead exposure on children's cognition and behavior, the level may be enough to put these children at risk for subsequent neurotoxicity.

Future Aims

We have focused on five micronutrients in this study. In future studies, it is important to examine the effects of other micro and macronutrients on lead exposure to get a complete understanding of the effects of nutritional factors on children's lead exposure. We faced a number of challenges in our efforts to capture and analyze dietary information in this population. Although, the nutrient database is widely used in Uruguayan population, there is a lack of studies on validity and reliability of the database. Future studies in this population should aim at validating this database in Uruguayan children to establish a nutrient database that can be used in research studies. It is also important to examine the dietary pattern and frequency of different food intakes of these children. This may in turn help to explain the nutrient deficiencies and if needed, to make changes in their diet. In future, replication of the study with biomarkers of nutrients is also warranted to confirm the findings of this study.

A better understanding of the source and extent of environmental exposure is warranted to detect the independent effect of nutritional factors on children's BLL. It is also important to assess the effects of nutritional factors on the toxicity caused by lead. For example, future studies should look at the effects of nutrient intakes on lead induced oxidative stress, which is thought to be one of the mechanisms by which lead exerts its toxicity. Finally, continued effort to establish a dietary recommendation for prevention of lead exposure and toxicity should be encouraged.

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