

**Auxology and Environmental Epidemiology: Lead Exposure,
Physical Growth and Maturation**

by

Myriam Carol Afeiche

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Doctoral Committee:

Professor Howard Hu, Chair
Professor Rita Loch-Caruso
Professor Karen E. Peterson
Assistant Professor Brisa N. Sánchez

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ABSTRACT

The theme of this dissertation is the potential impact of pre- and post-natal lead exposure on child growth and maturation. Maternal bone lead has been inversely associated with physical growth in infants and young children, but no study has examined whether this association persists throughout preschool years. In addition, lead exposure has been implicated as a possible contributing factor to delayed puberty in cross-sectional studies.

Taking advantage of a long-running birth cohort study in Mexico City that used maternal bone lead, a novel biomarker of prenatal lead exposure, we studied the association of lead with child weight trajectory from birth to 5 years, adjusting for postnatal lead exposure. Second, we identified critical windows of lead exposure susceptibility on children's height and body mass index (BMI) at 4 years of age. Third, we examined the association of prenatal lead exposure with puberty onset among a subset of 43 girls and 31 boys at ages 6 to 15 years.

Prenatal lead exposure was associated with a sustained decrease in girls' but not boys' weight over time, independent of postnatal lead exposure and adjusting for several confounders and predictors of child's weight. Second, we found that the most sensitive window of lead exposure on skeletal growth during development (measured by height) was infancy (birth to 24 months). Lead exposure was not associated with children's

attained BMI at 4 years of age. Preliminary findings from the follow-up study of youth aged 6 to 15 years do not support an association of prenatal lead exposure with pubertal onset, but these findings are limited by sample size.

Lead exposure at current environmental levels remains an important public health issue; it is associated with childhood weight and height deficits. Future research will investigate its association with onset of puberty in a larger sample from this cohort. The results of this research can inform future policies on the use and development of new and old pollutants.

CHAPTER I

Introduction

Theme

The theme of this dissertation is the potential impact of pre- and post-natal lead exposure on child physical growth and sexual maturation. Although lead is one of the best studied pollutants worldwide, its long-term effects on physical growth and maturation are not yet fully understood. This issue is of major public health importance as low-level environmental lead exposure remains common in both developed and developing countries (Tong, von Schirnding et al. 2000; Lanphear 2007; Bellinger 2008; Rossi 2008).

The objective of this research is to understand the association of lead with different aspects of physical growth in early childhood. Does lead depress growth; if so, which anthropometric measures (or indices) does it affect (weight, height, BMI)? What are the potential exposure windows of susceptibility of lead on growth in children before school entry? Is depressed growth followed by catch-up growth? Finally, is lead exposure associated with a delayed onset of puberty as suggested by a few epidemiological studies supported by evidence from animal models?

Lead exposure: a continuing public health problem

Effects and global sources of lead

Environmental lead exposure remains an important issue worldwide (Tong, von Schirnding et al. 2000; Gordon, Mackay et al. 2004; Rossi 2008). Its health effects are systemic and include reproductive, cardiovascular, renal, neurodevelopmental consequences (ATSDR 2007). The global burden of mental retardation due to lead has been estimated to represent 1% of the global burden of disease and 2% of the total cardiovascular disease burden (Fewtrell, Prüss-Üstün et al. 2004).

Even though worldwide lead exposure has decreased tremendously in the past decades (Pirkle, Brody et al. 1994; Hwang, Ko et al. 2004; Gulson, Mizon et al. 2006), mainly due to the phasing out of leaded gasoline, lead is still ubiquitous and often found in flaking lead-based paints (Mathee, Singh et al. 2009), candies (CDC 2002; Medlin 2004), cosmetics (Al-Ashban, Aslam et al. 2004), traditional lead-glazed ceramics used in the preparation and serving of food (Sabouraud, Coppéré et al. 2009), herbal remedies (Obi, Akunyili et al. 2006), canned foods and beverages (lead solder) (Maduabuchi, Nzegwu et al. 2006), toys (Greenway and Gerstenberger 2010), and can affect communities residing near smelters (Hegde, Sridhar et al. 2010) and battery recycling plants (Fuller 2009).

Sources of lead in Mexico City

Leaded gasoline is one of the environmental sources of lead to which our study cohort has been exposed. It was phased out in Mexico between 1991 and 1997 (Romieu, Palazuelos et al. 1994). Lead is still present in the bodies of the mothers in our cohort because of the long retention time of lead in bone (see section on Exposure assessment). Another prevalent ongoing source of lead exposure in Mexico City is the continued use of traditional lead-glazed ceramics in the preparation and serving of food (Romieu, Palazuelos et al. 1994; Chaudhary-Webb, Paschal et al. 2003). Exposure can occur through ingestion (from lead-glazed ceramics or leaded gasoline) or inhalation (leaded gasoline).

Environmental lead exposure, growth, and development

Auxology: the study of growth

Growth is determined by both environment and genetics (the relationship can be defined as an interaction) (Tanner 1990). Genetics (partly reflected by parental size) determines growth potential, while environment modulates how much of this growth potential is attained. Several environmental factors affect growth such as nutrition (Boulton, Garnett et al. 1999), chronic diseases in childhood, lifestyle (physical activity), and pollutants. Socio-economic status (SES) has been used as a proxy variable for these environmental factors. Child growth may mirror disparities in exposures and

environmental factors (Tanner 1990). Thus growth of children can be regarded as a “mirror of society” (Tanner 1987).

Growth can be defined as both physical growth (as measured by weight and height) as well as development (maturation). The outcomes examined in the following chapters are weight, height, BMI, and sexual maturation as measured by puberty onset.

Height reflects child nutrition and health status (chronic infections, illnesses etc). Weight-for-age is a useful indicator to evaluate if a child is underweight or severely underweight (WHO 1995). BMI is an index of weight relative to height (kg/m^2). It is used as a screening tool for overweight and obesity. High BMI correlates with high fat mass, but overall BMI does not accurately classify the percentage of fat across the distribution. In children, fat mass and therefore BMI change with age: BMI gets lower during the preschool years, then increases during adiposity rebound, and then continues to increase throughout adolescence. Percentiles of BMI-for-age are a better tool to screen for overweight and obese status. Percentiles of BMI-for-age are constructed by comparing the BMI of a specific child to the BMI distribution of a reference population at a specific age, within gender. As such, a BMI-for-age $\geq 85^{\text{th}}$ and $< 95^{\text{th}}$ percentile is an indicator for overweight; and a BMI-for-age $\geq 95^{\text{th}}$, an indicator for obesity. BMI-for-age $\geq 95^{\text{th}}$ percentile is a moderately sensitive and specific indicator of adiposity among children less than 18 years of age (Freedman and Sherry 2009).

Endocrinological regulation of postnatal physical growth

Stimulation of physical growth occurs through the pulsatile release of growth hormone by the anterior pituitary. The release of growth hormone in turn is coordinated by somatostatin, which acts as a growth inhibitor, and growth hormone releasing hormone (GHRH), which acts as a growth stimulant (Thorner, Vance et al. 1989). Somatostatin and GHRH are of hypothalamic origin. Synthesis and secretion of GH arises by GHRH binding to its receptor (Thorner, Vance et al. 1989).

Receptors for growth hormone are present on each cell of the human body. Through cell signaling in several organs and tissues, growth hormone leads to the production of somatomedin (also known as insulin-like growth factor, IGF). IGF-1 contributes to bone growth by increased protein synthesis and cell proliferation (Hindmarsh 1988; Tanner 1990). This hormone also controls somatostatin and GHRH through a feedback loop (Thorner, Vance et al. 1989).

Growth is also a result of gonadal steroids (namely, testosterone and estradiol) (Hindmarsh 1988). During puberty, the amplitude (but not frequency) of growth hormone pulses increases, and is primarily responsible for the adolescent height spurt (Tanner 1990).

Hormonal regulation of physical growth can be disrupted by lead. Animal studies have demonstrated that lead causes a decrease in circulating plasma IGF-1 levels (Ronis, Badger et al. 1998) and inhibits binding of growth hormone releasing hormone to its receptor (Camoratto, White et al. 1993).

Endocrinological control of puberty

Among humans, puberty occurs as a result of the activation of the hypothalamic-pituitary-gonadal (HPG) axis and hypothalamic-pituitary-adrenal (HPA) axis. Leptin has been hypothesized to be a permissive factor in the activation of the HPA and HPG axes as leptin levels increase during puberty (Styne 2001; Witchel and Plant 2004). Another suspected trigger of puberty is skeletal age (Styne and Grumbach 2003; Witchel and Plant 2004). Puberty begins when the central nervous system (CNS) signals the hypothalamus to discharge gonadotropin releasing hormone (GnRH) (Buck Louis, Gray et al. 2008). This hormone triggers the pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which stimulate the gonads (testes and ovaries) to produce androgen and estrogen. These sex hormones lead to pubertal changes and to the growth and maturation of the penis, pubic hair, testes, breasts, ovaries, and uterus. Similarly, adrenarche is initiated by the CNS, which stimulates the hypothalamus. Adrenocorticotropin releasing hormone (CRH) is discharged from the hypothalamus and triggers the pituitary to release adrenocorticotropin releasing hormone (ACTH). ACTH stimulates the adrenal cortex to release androstenedione and dehydroepiandrosterone (DHEA), which lead to the development of secondary sexual characteristics among males and females (such as pubic and axillary hair, and acne) (Buck Louis, Gray et al. 2008).

Skeletal development

Bones are comprised of three types of cells: osteoblasts, osteocytes, and osteoclasts. Osteoblasts are bone-forming cells involved in the synthesis of type I collagen as well as alkaline phosphatase. At the bone surface osteoblasts create new bone through mineralization using calcium and phosphate to form hydroxyapatite (Stini 1998). At this point, an element such as lead can be deposited in the remodeled bone (Stini 1998). Some of the osteoblasts then remain in cortical bone as osteocytes. Osteocytes work as multinucleate units to transport minerals, connect with nutrient capillaries, and communicate with surface osteoblasts (Shoback, Marcus et al. 2001). During the life course, bone remodeling is a dynamic interplay between bone resorption (osteoclasts) and bone formation (osteoblasts) (Shoback, Marcus et al. 2001).

During childhood and puberty, linear growth (bone elongation) occurs as a result of cartilage development at the ends of the diaphysis in the long bones the epiphyseal growth plate. Chondrocytes are cartilage-forming cells that produce collagen. Osteoblasts complete the final part of bone elongation by mineralization of collagen (Puzas, Sichel et al. 1992). Specific growth factors (such as insulin-like growth factor I (IGF-I), and transforming growth factor-beta1 (TGF- β 1)) regulate chondrocyte proliferation (Zuscik, Pateder et al. 2002). These growth factors are themselves controlled by signaling pathways (such as the activator protein 1 (AP-1)) essential for chondrocyte maturation and regulation. At the end of adolescence, when linear growth is complete, estrogen signals the close of the growth plate (Stini 1998; Chagin and Sävendahl 2009). Thus,

environmental insults affecting long bones occurring during development tend to result in permanent effects on attained height.

Lead and physical growth

Physical growth during childhood begins at birth with the product of fetal growth and is then shaped by the postnatal environment. Fetal and postnatal growth can be impacted by a number of factors, one of them being environmental insults such as lead. Altered growth rate might have significant consequences for the development of the child. Reduced growth rate, for example, might lead to a subsequent catch-up growth in childhood, which in turn, might be a risk factor for adult disease outcomes such as cardiovascular diseases, obesity, and hypertension (Eriksson, Forsén et al. 1999).

Catch-up growth has been observed among children exposed to high lead levels in utero but low levels postnatally. High lead exposure in utero has been associated with decrements in attained length at 15 months (Shukla, Bornschein et al. 1989); but lower subsequent lead levels were associated with catch-up from 15 to 33 months (Shukla, Dietrich et al. 1991). Schell and colleagues found that the effect of lead on several anthropometric indices at 12 months was both exposure-and time dependent and could be wholly or partially reversible (Schell, Denham et al. 2009).

Prenatal lead exposure and growth

In epidemiologic studies, prenatal lead exposure measured by maternal bone (patella and tibia) lead levels has been associated with smaller size at birth (weight,

length, and head circumference), weight attained at one month of age, and weight gain between birth and one month of age (Gonzalez-Cossío, Peterson et al. 1997; Sanín, Gonzalez-Cossío et al. 2001; Hernández-Avila, Peterson et al. 2002). Prenatal lead exposure measured by maternal blood lead levels during pregnancy or by cord blood lead levels also has been associated with decrements in physical growth of children in some studies (Shukla, Bornschein et al. 1989; Zentner, Rondo et al. 2006; Lamb, Janevic et al. 2008; Schell, Denham et al. 2009), but not in others (Greene and Ernhart 1991).

Postnatal lead exposure and growth

Postnatal lead exposure measured by child blood lead levels has been associated with delays in physical growth in some studies (Schwartz, Angle et al. 1986; Little, Snell et al. 1990; Frisancho and Ryan 1991; Shukla, Dietrich et al. 1991; Kafourou, Touloumi et al. 1997; Ballew, Khan et al. 1999; Little, Spalding et al. 2009), but not in others (Sachs and Moel 1989; Greene and Ernhart 1991).

These studies have examined population-wide lead exposure at levels around the U.S. Centers for Disease Control and Prevention (CDC) screening level of 10 µg/dL (CDC 1991) but not at occupational levels. Results of these studies, however, were limited by lead exposure measures. The biomarkers employed were not reflective of integrated lead exposure during pregnancy. Second, few studies incorporated repeated anthropometry through a longitudinal design. Finally, some studies were limited by their sample size.

Lead and puberty timing

Lead exposure in adolescence has been associated with delays in the timing of puberty in cross-sectional studies in nationally representative samples of U.S. females (Selevan, Rice et al. 2003; Wu, Buck et al. 2003; Gollenberg, Hediger et al. 2010), and among Akwesasne Mohawk girls in New York State (Denham, Schell et al. 2005); but not among inner-city U.S. females (Wolff, Britton et al. 2008). Among boys, one study in Russia suggests an association of lead with pubertal characteristics (Hauser, Sergeyev et al. 2008; Williams, Sergeyev et al. 2010). A major limitation of these studies is the cross-sectional design and the use of blood lead as a biomarker reflecting recent lead exposure.

Overall approach

Exposure assessment

Several biomarkers of prenatal lead exposure have been used in epidemiological studies as described above. Maternal bone lead has been demonstrated to be one of the most useful measures of cumulative fetal lead exposure; which in turn has been found in several studies to be best predictive of adverse postnatal outcomes such as intelligence quotient (IQ) (Gomaa, Hu et al. 2002) and linear growth (Hernández-Avila, Peterson et al. 2002).

The absorbed dose of lead is distributed in the blood, soft tissues (such as liver, kidneys, lungs, brain, spleen, muscles, and heart), and mineralizing tissues (such as bones

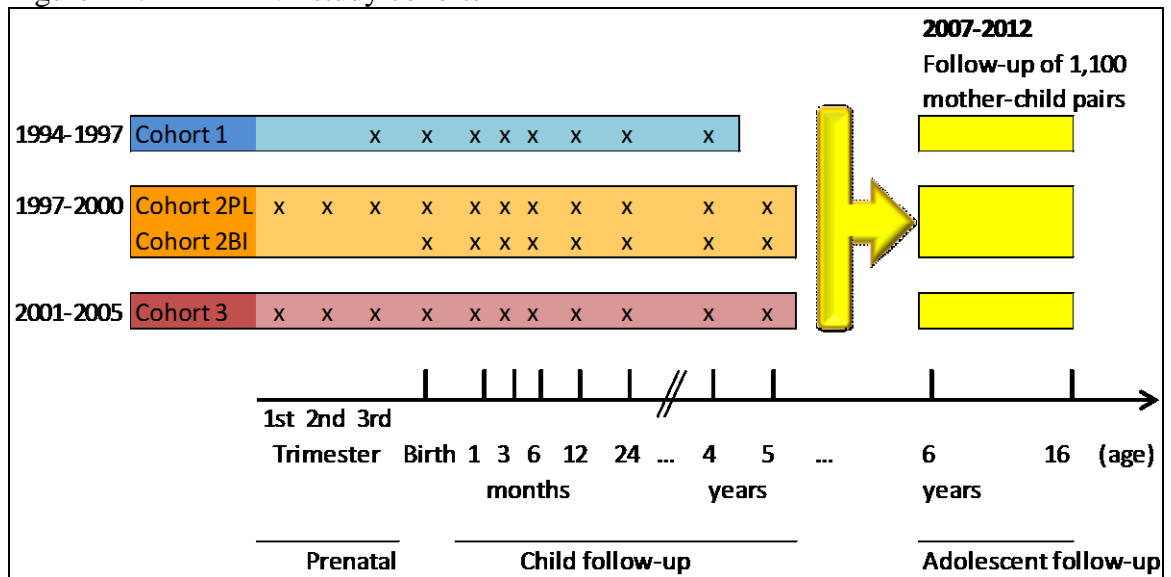
and teeth). However, most of the lead in the body is stored in the bones (90 to 95% among adults and 70 to 80% among children) for years to decades (Barry and Mossman 1970). Measurement of lead in bones represents historical exposure. The cortical bone (mid-tibial shaft) has more mineral collagen content than the trabecular bone (patella or knee cap) and lead accumulates slower in this bone component. Trabecular bone is more vascularized, and thus reflects more recent lead exposure than cortical bone (Hu, Milder et al. 1989; Hu, Rabinowitz et al. 1998). Due to their different physiology, lead metabolism behavior, and mineral turn-over rates, measurements were carried out in both the mid-tibial shaft (cortical bone) and the patella or knee cap (trabecular bone) in our study.

Most of the lead in blood is bound to red blood cells (Goyer 1990). Lead concentrations in the plasma reflect the amount of circulating lead available to cross the placenta by passive diffusion (Chuang, Schwartz et al. 2001). During pregnancy and lactation, the calcium needs of the developing fetus and infant are met by the increased resorption of maternal calcium stores in the bone and increased absorption in the gastrointestinal tract (Sowers, Corton et al. 1993). Because lead is stored in the bones, it is mobilized from the bones to plasma and crosses the placenta during pregnancy and lactation (Goyer 1990; Gulson, Jameson et al. 1997). Whole blood lead measurements reflect recent exposure. In fact, the half-life of lead in blood is about 35 days (Rabinowitz, Wetherill et al. 1976). Endogenous sources of lead – from the bones for example – also contribute to lead in blood. In this study, we used maternal bone lead to assess prenatal lead exposure and child blood lead to assess postnatal lead exposure.

Study population

The sample population for this thesis consists of 3 sequentially-enrolled longitudinal pooled birth cohorts recruited between 1994 and 2005 at maternity hospitals serving low-to-moderate income populations in Mexico City (Mexican Social Security Institute, Manuel Gea González Hospital, and National Institute of Perinatology). Starting in 2007, participants in the 3 birth cohorts were subsequently invited for a prospective follow-up after the child was at least 6 years of age. This study population is part of the ELEMENT (Early Life Exposures in Mexico to Environmental Toxicants) project (Figure I-1).

Figure I-1: ELEMENT study cohorts



In this dissertation, cohorts 2PL and 2BI are referred to as Cohort 2A and 2B respectively. The x's correspond to the time of study visit. For example, Cohort 1 mothers were recruited during the 3rd trimester of pregnancy. Children in Cohort 1 were followed up until 4 years of age whereas those in Cohorts 2 (PL and BI) and 3 until 5 years of age.

Cohort 1 was a randomized control trial of the effect of calcium supplementation on blood lead levels during lactation. Study methods have been extensively described elsewhere (Gonzalez-Cossío, Peterson et al. 1997; Téllez-Rojo, Hernández-Avila et al. 2002; Hernández-Avila, Gonzalez-Cossío et al. 2003). Briefly, 617 mother-infant pairs were recruited between 1994 and 1995 and followed until 1997. Cohort 2 comprised two groups, the first (Cohort 2A, n=327) recruited pre-pregnancy and followed between 1997 and 2000, and the second (Cohort 2B, n=462) was recruited during pregnancy and followed between 1999 and 2000. Data collection methods have been described elsewhere (Téllez-Rojo, Hernandez-Avila et al. 2004; Hu, Téllez-Rojo et al. 2006). The 557 mothers in Cohort 3 were enrolled in a randomized trial of calcium supplementation during pregnancy (Ettinger, Lamadrid-Figueroa et al. 2009) and their children followed from 2000 to 2007.

Similar exclusion criteria were applied to the 3 cohorts, such as, living outside of Mexico City, a physician's diagnosis of current multiple pregnancies, gestational diabetes, seizure disorder requiring daily medications except for other criteria described elsewhere (Gonzalez-Cossío, Peterson et al. 1997; Téllez-Rojo, Hernandez-Avila et al. 2004; Ettinger, Lamadrid-Figueroa et al. 2009), as shown in Table I-1.

Table I-1: Exclusion criteria by cohort

Exclusion criteria	Cohort			
	1	2A	2B	3
No intention to breastfeed	x			
Living outside of Mexico City	x	x	x	x
Having plans to leave Mexico City in the following 5 years		x	x	
Not trying to become pregnant or no positive B-human chorionic gonadotropin test		x		
Conditions related to calcium metabolism and requirements	x			
Not agreeing to participate in follow-up		x	x	x
Multiple fetuses/ a physician's diagnosis of current multiple pregnancy	x	x	‡	x
Preeclampsia, or pregnancy-related hypertensive disorders	x	x	x	x
Psychiatric disease	x	x		
Kidney or cardiac disease	x	x		x
Gestational diabetes	x	x	x	x
History of repeated urinary infections	x	x		
Family or personal history of kidney stone formation	x			
Seizure disorder requiring daily medications	x	x	x	x
Use of corticosteroids	x			
Blood pressure >140 mm Hg systolic or > 90 mm Hg diastolic	x			
Premature neonates (<37 weeks) or newborns with low birth weight (<2000 g)	x			
Pregnancy of more than 20 weeks' gestation		x	‡	
Pregnancy of more than 14 weeks' gestation			‡	x
Daily consumption of alcoholic beverages		x		x
Addiction to illegal drugs		x		x
Continuous use of prescription drugs		x		x
Not intending to become pregnant	‡	x		x
Presenting with a high-risk pregnancy		x	x	x
Infant with an Apgar score at 5 minutes of 6 or under	x			
Conditions requiring infant placement in a neonatal intensive care unit	x			

‡ Refers to not applicable.

The 3 birth cohorts were pooled for the purposes of this study. Although each cohort was studied for distinct specific aims, combining the three cohorts was possible

through the use of common methods for recruiting subjects, and common measures of pre-and post-natal lead exposure, covariates, and anthropometry. The published manuscripts that will result from this dissertation are our research group's first study that combines all three cohorts.

Anthropometry

Weight and length of nude newborns were measured within 12 hours of delivery by experienced obstetric nurses. At subsequent study visits, children's weight and height were collected by trained staff (Lohman, Roche et al. 1988) using standard protocols (Habicht 1974). Calibrated beam scales (Oken, Model TD16, Naucalpan, México) were used and read to the nearest 10 grams (g). Maternal height was obtained at 1-month postpartum using professional scales (PAME, Puebla, Puebla) read to the nearest millimeter (mm). Maternal calf circumference was used as a proxy for maternal body size, consistent with previous research (Gonzalez-Cossío, Peterson et al. 1997). Maternal calf circumference was measured at 1-month postpartum with plastic-covered fabric measuring tapes read to the nearest mm. As described elsewhere (Gonzalez-Cossío, Peterson et al. 1997), standardization exercises were performed until the project staff reached imprecision errors (3 mm for calf circumferences and 2.2 mm for height) equal to or below those reported by Lohman and coworkers (Lohman, Roche et al. 1988).

Thesis overview

Chapter II investigates the association of maternal bone lead with repeated measures of weight from birth to 5 years of age, controlling for child blood lead. Taking advantage of the longitudinal study design, we are able to disentangle the association of both pre- and post-natal lead exposure with attained weight over a period of 5 years.

Chapter III explores windows of lead exposure susceptibility during growth through the use of lead exposure histories. The identified windows include prenatal, infancy (from birth to 24 months), and early childhood (from 30 to 48 months). The growth outcomes investigated are height and BMI at 4 years of age.

The aim of Chapter IV is to describe the study design and explore the association between prenatal lead exposure and puberty onset among a subset of the same children followed-up at 6 to 15 years of age.

Conclusions and future directions are described in Chapter V.

References

- Al-Ashban, R. M., M. Aslam, et al. (2004). "Kohl (surma): a toxic traditional eye cosmetic study in Saudi Arabia." Public Health **118**(4): 292-298.
- ATSDR (2007). Toxicological Profile for Lead. U.S. Department of Health and Human Services. Public Health Service. Atlanta, GA.
- Ballew, C., L. K. Khan, et al. (1999). "Blood lead concentration and children's anthropometric dimensions in the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994." The Journal of Pediatrics **134**(5): 623-30.
- Barry, P. S. and D. B. Mossman (1970). "Lead concentrations in human tissues." British Journal of Industrial Medicine **27**(4): 339-51.
- Bellinger, D. C. (2008). "Neurological and behavioral consequences of childhood lead exposure." PLoS medicine **5**(5): e115-e115.
- Boulton, T. J., S. P. Garnett, et al. (1999). "Nutrition in early life: somatic growth and serum lipids." Annals of Medicine **31 Suppl 1**: 7-12.
- Buck Louis, G. M., L. E. Gray, Jr., et al. (2008). "Environmental factors and puberty timing: expert panel research needs." Pediatrics **121 Suppl 3**: S192-207.
- Camoratto, A. M., L. M. White, et al. (1993). "Effect of exposure to low level lead on growth and growth hormone release in rats." Toxicology **83**(1-3): 101-114.
- CDC (1991). Preventing lead poisoning in young children. Atlanta, GA, US Department of Health and Human Services.
- CDC (2002). "Childhood lead poisoning associated with tamarind candy and folk remedies--California, 1999-2000." MMWR. Morbidity and Mortality Weekly Report **51**(31): 684-686.
- Chagin, A. S. and L. Sävendahl (2009). "Genes of Importance in the Hormonal Regulation of Growth Plate Cartilage." Hormone Research in Paediatrics **71**(Suppl. 2): 41-47.
- Chaudhary-Webb, M., D. C. Paschal, et al. (2003). "Determining lead sources in Mexico using the lead isotope ratio." Salud Pública de México **45**: 183-188.

Chuang, H. Y., J. Schwartz, et al. (2001). "Interrelations of lead levels in bone, venous blood, and umbilical cord blood with exogenous lead exposure through maternal plasma lead in peripartum women." Environmental Health Perspectives **109**(5): 527-32.

Denham, M., L. M. Schell, et al. (2005). "Relationship of lead, mercury, mirex, dichlorodiphenyldichloroethylene, hexachlorobenzene, and polychlorinated biphenyls to timing of menarche among Akwesasne Mohawk girls." Pediatrics **115**(2): e127-34.

Eriksson, J. G., T. Forsén, et al. (1999). "Catch-up growth in childhood and death from coronary heart disease: longitudinal study." British Medical Journal **318**(7181): 427-431.

Ettinger, A. S., H. Lamadrid-Figueroa, et al. (2009). "Effect of calcium supplementation on blood lead levels in pregnancy: a randomized placebo-controlled trial." Environmental Health Perspectives **117**(1): 26-31.

Fewtrell, L. J., A. Prüss-Üstün, et al. (2004). "Estimating the global burden of disease of mild mental retardation and cardiovascular diseases from environmental lead exposure." Environmental Research **94**(2): 120-133.

Freedman, D. S. and B. Sherry (2009). "The validity of BMI as an indicator of body fatness and risk among children." Pediatrics **124 Suppl 1**: S23-34.

Frisancho, A. R. and A. S. Ryan (1991). "Decreased stature associated with moderate blood lead concentrations in Mexican-American children." The American Journal of Clinical Nutrition **54**(3): 516-9.

Fuller, R. (2009). "Lead Exposures from Car Batteries --A Global Problem." Environmental Health Perspectives **117**(12).

Gollenberg, A. L., M. L. Hediger, et al. (2010). "Association Between Lead and Cadmium and Reproductive Hormones in Peripubertal U.S. Girls." Environmental Health Perspectives.

Gomaa, A., H. Hu, et al. (2002). "Maternal bone lead as an independent risk factor for fetal neurotoxicity: a prospective study." Pediatrics **110**(1 Pt 1): 110-8.

Gonzalez-Cossío, T., K. E. Peterson, et al. (1997). "Decrease in birth weight in relation to maternal bone-lead burden." Pediatrics **100**(5): 856-62.

Gordon, B., R. Mackay, et al. (2004). Inheriting the world: the atlas of children's health and the environment. Geneva, World Health Organization.

- Goyer, R. A. (1990). "Transplacental transport of lead." Environmental Health Perspectives **89**: 101-5.
- Greene, T. and C. B. Ernhart (1991). "Prenatal and preschool age lead exposure: relationship with size." Neurotoxicology and Teratology **13**(4): 417-27.
- Greenway, J. and S. Gerstenberger (2010). "An evaluation of lead contamination in plastic toys collected from day care centers in the las vegas valley, nevada, USA." Bulletin of Environmental Contamination and Toxicology **85**(4): 363-366.
- Gulson, B., K. Mizon, et al. (2006). "Changes in manganese and lead in the environment and young children associated with the introduction of methylcyclopentadienyl manganese tricarbonyl in gasoline--preliminary results." Environmental Research **100**(1): 100-114.
- Gulson, B. L., C. W. Jameson, et al. (1997). "Pregnancy increases mobilization of lead from maternal skeleton." The Journal of Laboratory and Clinical Medicine **130**(1): 51-62.
- Habicht, J. P. (1974). "[Standardization of quantitative epidemiological methods in the field]." Boletín de la Oficina Sanitaria Panamericana **76**(5): 375-384.
- Hauser, R., O. Sergeev, et al. (2008). "Association of blood lead levels with onset of puberty in Russian boys." Environmental Health Perspectives **116**(7): 976-980.
- Hegde, S., M. Sridhar, et al. (2010). "Relating tooth- and blood-lead levels in children residing near a zinc-lead smelter in India." International Journal of Paediatric Dentistry **20**(3): 186-192.
- Hernández-Avila, M., T. Gonzalez-Cossío, et al. (2003). "Dietary calcium supplements to lower blood lead levels in lactating women: a randomized placebo-controlled trial." Epidemiology **14**(2): 206-12.
- Hernández-Avila, M., K. E. Peterson, et al. (2002). "Effect of maternal bone lead on length and head circumference of newborns and 1-month-old infants." Archives of Environmental Health **57**(5): 482-8.
- Hindmarsh, P. C. (1988). Endocrinological regulation of post-natal growth. The Cambridge encyclopedia of human growth and development. S. J. Ulijaszek, F. E. Johnston and M. A. Preece. Cambridge, UK, Cambridge University Press.

Hu, H., F. L. Milder, et al. (1989). "X-ray fluorescence: issues surrounding the application of a new tool for measuring burden of lead." Environmental Research **49**(2): 295-317.

Hu, H., M. Rabinowitz, et al. (1998). "Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms." Environmental Health Perspectives **106**(1): 1-8.

Hu, H., M. M. Téllez-Rojo, et al. (2006). "Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development." Environmental Health Perspectives **114**(11): 1730-5.

Hwang, Y.-H., Y. Ko, et al. (2004). "Transition of cord blood lead level, 1985-2002, in the Taipei area and its determinants after the cease of leaded gasoline use." Environmental Research **96**(3): 274-282.

Kafourou, A., G. Touloumi, et al. (1997). "Effects of lead on the somatic growth of children." Archives of Environmental Health **52**(5): 377-83.

Lamb, M. R., T. Janevic, et al. (2008). "Environmental lead exposure, maternal thyroid function, and childhood growth." Environmental Research **106**(2): 195-202.

Lanphear, B. P. (2007). "The conquest of lead poisoning: a Pyrrhic victory." Environmental Health Perspectives **115**(10): A484-A485.

Little, B. B., L. M. Snell, et al. (1990). "Blood lead levels and growth status of children." American Journal of Human Biology **2**(3): 265-269.

Little, B. B., S. Spalding, et al. (2009). "Blood lead levels and growth status among African-American and Hispanic children in Dallas, Texas--1980 and 2002: Dallas Lead Project II." Annals of Human Biology **36**(3): 331-341.

Lohman, T. G., A. F. Roche, et al. (1988). Anthropometric standardization reference manual. Champaign, IL, Human Kinetics Books.

Maduabuchi, J. M. U., C. N. Nzegwu, et al. (2006). "Lead and cadmium exposures from canned and non-canned beverages in Nigeria: a public health concern." The Science of the Total Environment **366**(2-3): 621-626.

Mathee, A., E. Singh, et al. (2009). "Lead-based paint on playground equipment in public children's parks in Johannesburg, Tshwane and Ekurhuleni." South African Medical Journal **99**(11): 819-821.

- Medlin, J. (2004). "Sweet candy, bitter poison." Environmental Health Perspectives **112**(14): A803.
- Obi, E., D. N. Akunyili, et al. (2006). "Heavy metal hazards of Nigerian herbal remedies." The Science of the Total Environment **369**(1-3): 35-41.
- Pirkle, J. L., D. J. Brody, et al. (1994). "The decline in blood lead levels in the United States. The National Health and Nutrition Examination Surveys (NHANES)." JAMA **272**(4): 284-291.
- Puzas, J. E., M. J. Sickel, et al. (1992). "Osteoblasts and chondrocytes are important target cells for the toxic effects of lead." Neurotoxicology **13**(4): 783-8.
- Rabinowitz, M. B., G. W. Wetherill, et al. (1976). "Kinetic analysis of lead metabolism in healthy humans." Journal of Clinical Investigation **58**(2): 260-270.
- Romieu, I., E. Palazuelos, et al. (1994). "Sources of lead exposure in Mexico City." Environmental Health Perspectives **102**(4): 384-9.
- Ronis, M. J., T. M. Badger, et al. (1998). "Endocrine mechanisms underlying the growth effects of developmental lead exposure in the rat." Journal of Toxicology and Environmental Health Part A **54**(2): 101-20.
- Rossi, E. (2008). "Low level environmental lead exposure--a continuing challenge." The Clinical Biochemist Reviews **29**(2): 63-70.
- Sabouraud, S., B. Coppéré, et al. (2009). "[Environmental lead poisoning from lead-glazed earthenware used for storing drinks]." La Revue de Médecine Interne **30**(12): 1038-1043.
- Sachs, H. K. and D. I. Moel (1989). "Height and weight following lead poisoning in childhood." American Journal of Diseases of Children **143**(7): 820-2.
- Sanín, L. H., T. Gonzalez-Cossío, et al. (2001). "Effect of maternal lead burden on infant weight and weight gain at one month of age among breastfed infants." Pediatrics **107**(5): 1016-23.
- Schell, L., M. Denham, et al. (2009). "Growth of infants' length, weight, head and arm circumferences in relation to low levels of blood lead measured serially." American Journal of Human Biology **21**(2): 180-187.

Schwartz, J., C. Angle, et al. (1986). "Relationship between childhood blood lead levels and stature." Pediatrics **77**(3): 281-8.

Selevan, S. G., D. C. Rice, et al. (2003). "Blood lead concentration and delayed puberty in girls." New England Journal of Medicine **348**(16): 1527-36.

Shoback, D., R. Marcus, et al. (2001). Mineral metabolism and metabolic bone disease. Basic & clinical endocrinology. F. S. Greenspan and D. Gardner. New York, Lange Medical Books/McGraw-Hill.

Shukla, R., R. L. Bornschein, et al. (1989). "Fetal and infant lead exposure: effects on growth in stature." Pediatrics **84**(4): 604-12.

Shukla, R., K. N. Dietrich, et al. (1991). "Lead exposure and growth in the early preschool child: a follow-up report from the Cincinnati Lead Study." Pediatrics **88**(5): 886-92.

Sowers, M., G. Corton, et al. (1993). "Changes in bone density with lactation." JAMA **269**(24): 3130-3135.

Stini, W. A. (1998). Skeletal development. The Cambridge encyclopedia of human growth and development. S. J. Ulijaszek, F. E. Johnston and M. A. Preece. Cambridge, Cambridge University Press.

Styne, D. (2001). Puberty. Basic & clinical endocrinology. F. S. Greenspan and D. Gardner. New York, Lange Medical Books/McGraw-Hill.

Styne, D. and M. M. Grumbach (2003). Puberty: Ontogeny, Neuroendocrinology, Physiology, and Disorders. Williams textbook of endocrinology. R. H. Williams and P. R. Larsen. Philadelphia, Saunders.

Tanner, J. M. (1987). "Growth as a mirror of the condition of society: secular trends and class distinctions." Acta Paediatrica Japonica **29**(1): 96-103.

Tanner, J. M. (1990). The endocrinology of growth. Foetus into man: physical growth from conception to maturity. Cambridge, Massachusetts, Harvard University Press.

Tanner, J. M. (1990). Foetus into man: physical growth from conception to maturity. Cambridge, Massachusetts., Harvard University Press.

Téllez-Rojo, M. M., M. Hernández-Avila, et al. (2002). "Impact of breastfeeding on the mobilization of lead from bone." American Journal of Epidemiology **155**(5): 420-8.

Télléz-Rojo, M. M., M. Hernandez-Avila, et al. (2004). "Impact of bone lead and bone resorption on plasma and whole blood lead levels during pregnancy." American Journal of Epidemiology **160**(7): 668-78.

Thorner, M. O., M. L. Vance, et al. (1989). Growth hormone and growth hormone releasing hormone. The Physiology of human growth. J. M. Tanner, M. A. Preece and B. Society for the Study of Human. Cambridge, Cambridge University Press.

Tong, S., Y. E. von Schirnding, et al. (2000). "Environmental lead exposure: a public health problem of global dimensions." Bulletin of the World Health Organization **78**(9): 1068-1077.

WHO (1995). Physical Status: The Use and Interpretation of Anthropometry. Report of a WHO Expert Committee, Geneva, Switzerland: World Health Organization.

Williams, P., O. Sergeev, et al. (2010). "Blood lead levels and delayed onset of puberty in a longitudinal study of Russian boys." Pediatrics **125**(5): e1088-e1096.

Witchel, S. F. and T. M. Plant (2004). Puberty: Gonadarche and Adrenarche. Yen and Jaffe's reproductive endocrinology: physiology, pathophysiology, and clinical management. S. S. C. Yen, J. F. Strauss and R. L. Barbieri. Philadelphia, Elsevier Saunders.

Wolff, M. S., J. A. Britton, et al. (2008). "Environmental exposures and puberty in inner-city girls." Environmental Research **107**(3): 393-400.

Wu, T., G. M. Buck, et al. (2003). "Blood lead levels and sexual maturation in U.S. girls: the Third National Health and Nutrition Examination Survey, 1988-1994." Environ Health Perspect **111**(5): 737-41.

Zentner, L. E., P. H. Rondo, et al. (2006). "Lead contamination and anthropometry of the newborn baby." Journal of Tropical Pediatrics **52**(5): 369-71.

Zuscik, M. J., D. B. Pateder, et al. (2002). "Lead alters parathyroid hormone-related peptide and transforming growth factor-beta1 effects and AP-1 and NF-kappaB signaling in chondrocytes." Journal of Orthopaedic Research **20**(4): 811-8.

CHAPTER II

Prenatal Lead Exposure and Weight of 0 to 5 Year-Old Children in Mexico City

Abstract

Background:

Cumulative prenatal lead exposure as measured by maternal bone lead burden has been associated with smaller weight of offspring at birth and 1 month of age, but no study has examined whether this effect persists into early childhood. We investigated the association of maternal bone lead, a biomarker of cumulative prenatal lead exposure, with children's attained weight over time from birth to 5 years of age.

Methods:

Children were weighed at birth and at several intervals up until 12 months of age and every 6 months from 12 to 60 months. Maternal tibia and patella lead were measured at 1-month postpartum using *in vivo* K-X-ray fluorescence. Varying coefficient models with random effects were used to assess the effect of tibia and patella lead on weight trajectories of 522 males and 477 females born between 1994 and 2005 in Mexico City.

Results:

After controlling for breastfeeding duration, maternal anthropometry, and socio-demographic characteristics, a 1 standard deviation increase in patella lead was

associated with a 130.9 g decrease in weight (95% CI= -227.4 to -34.4) among females and a 13.0 g non-significant increase in weight among males (95% CI= -73.7 to 99.9) at 5 years of age. These effects were similar after controlling for concurrent blood lead levels between birth and 5 years (171.6 g decrease in weight among females, 95% CI= -275.2 to -68.0, and a non-significant 35.0 g among males, 95% CI= -132.4 to 62.3 at 5 years of age).

Conclusion:

Maternal post-partum bone lead was associated with lower weight over time among female but not male children up to 5 years of age.

Introduction

The developmental origins hypothesis stipulates that constraints on fetal growth can have lasting impact on postnatal growth and related chronic diseases (Eriksson, Forsén et al. 1999; Barker 2006). Although lead levels have decreased in the last few decades in the United States (Jones, Homa et al. 2009) and in other countries (Hwang, Ko et al. 2004; Gulson, Mizon et al. 2006), environmental lead continues to be a public health problem that may restrict both fetal and child growth (Hernández-Avila, Peterson et al. 2002). Maternal lead exposure during pregnancy has been related to fetal growth, as reflected by infant size at birth (Andrews, Savitz et al. 1994; Jelliffe-Pawlowski, Miles et al. 2006). Prenatal lead exposure measured by maternal blood lead has been associated with decreases in children's weight-for-age z-scores at 6 months (Schell, Denham et al. 2009). Perinatal blood lead exposure has been associated with decrements in weight-for-age, weight-for-length, and length at 6 and 15 months of age followed by a catch-up growth at 12 and 33 months respectively if postnatal exposure was low (Shukla, Bornschein et al. 1989; Shukla, Dietrich et al. 1991; Schell, Denham et al. 2009).

The association of blood lead levels (pre- and post-natal) with child height has been more consistent than with weight (Andrews, Savitz et al. 1994; Peterson, Salganik et al. 2004; Schell, Gallo et al. 2006), although inferences from some studies have been limited by their cross-sectional design (Schwartz, Angle et al. 1986; Frisancho and Ryan 1991; Kafourou, Touloumi et al. 1997; Ballew, Khan et al. 1999). Less attention has been

paid to prenatal lead exposure as measured by a cumulative marker of maternal lead burden and its relationship to child growth over time. Mobilization of lead in the bones is a source of endogenous exposure after external lead exposure has ceased (Gulson, Jameson et al. 1997; Gulson, Pounds et al. 1999), especially during pregnancy and lactation, when the calcium needs of the developing fetus and infant are partially met by the resorption of maternal calcium stores in the bone.

Maternal bone lead burden has been associated with a decrease in birth weight (Gonzalez-Cossío, Peterson et al. 1997) and lower weight gain from birth to 1 month of age and lower attained weight at 1 month (Sanín, Gonzalez-Cossío et al. 2001). However, no study has examined whether the association of maternal bone lead with weight persists into early childhood.

The present study aims to discern the longitudinal effect of prenatal lead exposure as measured by maternal bone lead burden on weight over time from birth to 5 years adjusted for postnatal lead exposure. We hypothesized that prenatal lead exposure would lead to decreases in repeated measures of weight over time.

Materials and Methods

Study Population

Similar exclusion criteria were applied to the three cohorts, such as: living outside of Mexico City, a physician's diagnosis of current multiple pregnancies, gestational diabetes, and seizure disorder requiring daily medications (Gonzalez-Cossío, Peterson et

al. 1997; Téllez-Rojo, Hernandez-Avila et al. 2004; Ettinger, Lamadrid-Figueroa et al. 2009). For purposes of this study, we applied additional eligibility criteria: missing information on child weight and length at birth, date of birth, gender, gestational age; and maternal characteristics for example age at delivery, calf circumference, height, educational level, marital status, parity, breastfeeding duration, and patella lead (N=378 excluded). Also excluded were low birth weight (<2500 g, N=77) and premature (<37 weeks of gestation, N=97) neonates in order to minimize bias in the results due to postnatal catch-up growth characteristic of these infants (Binkin, Yip et al. 1988). Subjects with extreme outliers of child anthropometry (weight and height, N=58), maternal anthropometry (calf circumference and height, N=5), and lead measures (tibia and patella bone lead, and log-transformed child blood lead, N=34) were excluded from further analysis. Because participants could have been excluded for multiple reasons, the final analytic sample was comprised of 1000 mothers of 523 males and 477 females.

At time of recruitment, mothers in each cohort were given detailed information about the study procedures, provided information on ways to minimize lead exposure and signed a written letter of informed consent. The research protocol was approved by the Ethics and Research Committees of the partnering institutions including the National Institute of Public Health of Mexico, the Harvard School of Public Health, the Brigham and Women's Hospital, the University of Michigan School of Public Health, and the participating hospitals.

Study Visits

Participants were interviewed after delivery for all cohorts at the maternity hospitals; and at the research clinic when the child was 1, 5, and 7 months of age (cohort 1) and 3 and 6 months of age (cohorts 2A, 2B, and 3). Study visits were then scheduled at age 12 months and every 6 months thereafter until age of 48 months for cohort 1 and 60 months for the other cohorts. Maternal and child characteristics were collected through questionnaires administered by research staff.

Gestational age was estimated from the date of the last menstrual period recalled by the mother. At each study visit, mothers were asked whether they were breastfeeding. Because study visits were scheduled at different timepoints for each cohort, breastfeeding status was assessed at 7 months for cohort 1 and at 6 months for cohorts 2A, 2B, and 3. From here on we will refer to this variable as breastfeeding for 6 months.

Exposure Assessment

Bone Lead Measurements

In vivo maternal bone lead measurements (μg lead per/g bone mineral) were taken at 1 month of delivery (+/- 5 days) at two bone sites, the mid-tibial shaft (cortical bone) and the patella (trabecular bone) using K X-ray fluorescence (K-XRF). Bone lead was measured non-invasively using a spot-source ^{109}Cd K-XRF instrument constructed at Harvard University and installed in a research facility in the American British Cowdray Medical Center in Mexico City. The physical principles, technical specifications, and

validation of this and other similar K-XRF instruments have been described in detail elsewhere (Hu, Milder et al. 1989; Burger, Milder et al. 1990; Hu, Milder et al. 1991; Aro, Todd et al. 1994; Hu, Aro et al. 1995; Hu, Rabinowitz et al. 1998). When excited with a ^{109}Cd gamma-ray source, lead atoms in the target tissue emit fluorescent photons. This X-ray signal is then analyzed by a computer and provides a bone lead measurement. Negative measurements are generated when the true bone lead value is below the limit of detection (2 ppb).

Blood Lead Measurements

Whole blood was collected from children in trace metal-free tubes (BD Vacutainer® #367734, Becton-Dickinson, Franklin Lakes, NJ) and sampling was conducted at each interview by trained staff using standard protocols. Blood lead samples were analyzed at the metals laboratory of the American British Cowdray Hospital in Mexico City using a graphite furnace atomic absorption spectroscopy (GFAAS) (Perkin-Elmer 3000, Chelmsford, MA, USA). The units of measurement are in micrograms per deciliter ($\mu\text{g}/\text{dL}$). The instrument precision is within 1 $\mu\text{g}/\text{dL}$ and the limit of detection is less than 1 $\mu\text{g}/\text{dL}$. Blood lead measures were not collected at every visit due to child or maternal refusal, child inability to give blood, child being sick, or not scheduling a blood lead measure at that visit.

As described elsewhere (Gonzalez-Cossío, Peterson et al. 1997), external blinded quality control samples were analyzed by the Maternal & Child Health Bureau (MCHB) and the Wisconsin State Laboratory of Hygiene (WSLH) Cooperative Blood Lead

Proficiency Testing Program (PBPTP), and demonstrated adequate precision and accuracy determinations at the American British Cowdray Hospital, ($r= 0.98$; mean difference of $0.71 \mu\text{g/dL}$ compared with MCHB and WSLH PBPTP blanks and spikes).

Statistical analyses

Univariate and Bivariate Analyses

Descriptive statistics and distributions were examined. Extreme outliers of maternal and child anthropometry and lead measures were identified using the generalized extreme studentized deviation (ESD) method (Rosner 1983). T-test for continuous variables and chi-squared test for categorical variables were carried out to compare differences between participants with complete information and participants excluded due to the additional study eligibility criteria.

Covariates were chosen *a priori* based on biological relevance and were included in the analyses as known predictors of child's weight or potential confounders of the association between prenatal lead exposure and child weight over time. These consisted of: maternal anthropometry (height and calf circumference), socio-demographic characteristics (education, marital status, parity, age at delivery), lifestyle (breastfeeding), and child characteristics (such as gestational age, cohort, and repeated measures of child height) (Gonzalez-Cossío, Sanín et al. 1998). Maternal socio-demographic characteristics such as education and marital status have been correlated with birth outcomes and are reflective of available resources and prenatal nutrition (Cogswell and Yip 1995; Kramer, Seguin et al. 2000). Young and old maternal age have been associated with low birth

weight and other adverse birth outcomes (Kramer 1987). Parity was included in the model to account for bone lead mobilization due to previous pregnancies (Hernández-Avila, Gonzalez-Cossío et al. 1996) and for size at birth (children from primiparous women tend to be smaller at birth) (Kramer 1987). Breastfed children have a different growth pattern than formula-fed children (Dewey, Heinig et al. 1993; Garza and De Onis 1999; de Onis, Onyango et al. 2006; de Onis, Garza et al. 2007). In addition, breastfeeding is an important covariate as research has demonstrated that lead can be excreted into breast milk, exposing the fetus to lead (Ettinger, Téllez-Rojo et al. 2004; Ettinger, Téllez-Rojo et al. 2004).

The correlations among pairs of predictors were examined to assess potential multicollinearity. Linear regression models were performed to examine the bivariate relationship between each variable and our primary exposure variables (patella and tibia bone lead measures).

Outcome Models

Given the repeated measures of child weight over time, varying coefficient models with random effects were used to quantify the multivariable association of child, maternal, and lead characteristics with weight over time:

$$y_{i(t)} = \beta_0(t) + \beta_1(t)X_i + b_i(t) + \varepsilon_{it}$$

The attained weight by child i at age t is denoted by $y_{i(t)}$; $\beta_0(t)$ is the average weight for the sample population at time t ; $\beta_1(t)$ represents changes to the average weight trajectory associated with covariate X ; $b_i(t)$ is a subject-specific random effect; and ε_{it} is the random error. The terms $\beta_0(t)$ and $\beta_1(t)$ are time varying coefficients and are smooth functions of

child's age in months. For example, $\beta_0(t)$ evaluated at $t=0$, $\beta_0(0)$, would be the weight at birth for the average individual; similarly, $\beta_1(0)$ would be the average difference in weight at birth associated with a 1 unit increase in X . Hence, the model allows the association between weight and the covariate X to change with child's age (i.e., a time-varying coefficient), and be evaluated at particular times during child's growth (e.g., at 1 month after birth).

Multivariable outcome models were fitted separately for males and females to assess the effect of prenatal lead exposure as measured by levels in maternal patella and tibia bone on child weight trajectories, adjusted for potential confounders. We define "weight trajectories" as the repeated measures of child's attained weight at each age (in months) between birth and 5 years of age. The intercept and child's age were treated as random effects. All other variables were fixed effects and were centered at their mean. Models were examined separately for tibia and patella because of collinearity between the two bone lead measures; bone lead measures were standardized.

We constructed the predicted weight trajectories for children 2 standard deviations (SD) above and below the sample mean of bone lead by estimating $\hat{\beta}_0(t) \pm 2\hat{\beta}_{lead}(t)$, this can be obtained from model output using the `predict` function in R. Data were analyzed using SAS 9.2 (Cary, NC, SAS Institute Inc.) and R 2.11.1 (The R Foundation for Statistical Computing).

Results

Of the 1504 mother-infant pairs, 1000 participants had complete information at baseline (290 in cohort 1; 150 in 2A; 300 in 2B; and 260 in 3). There were statistically significant differences between those participants included in the analyses and those excluded for the following variables: gestational age, weight at birth, marital status, breastfed for 6 months, and patella and tibia bone lead (Table II-1). The differences observed for gestational age and weight at birth reflected this study's eligibility criteria of excluding premature and low birth weight infants. Bone lead differences were found due to the exclusion of outliers from the analytical sample.

In the pooled analysis, participating mothers had a mean age at delivery of 26 years. Most mothers were married (70%), had on average 10+ years of education, and 39% were primiparous. Mean maternal bone lead at 1-month postpartum across all three cohorts was 10.4 $\mu\text{g Pb/g}$ (SD= 11.8) for patella and 8.6 $\mu\text{g Pb/g}$ (SD= 9.7) for tibia; the correlation was 0.34 ($P<0.001$) between the two bone lead measures. Bone lead levels differed by cohort: 13.6 $\mu\text{g Pb/g}$ (SD= 14.5) for Cohort 1; 12.9 $\mu\text{g Pb/g}$ (SD= 11.1) for Cohort 2A; 8.6 $\mu\text{g Pb/g}$ (SD= 9.9) for Cohort 2B; 7.2 $\mu\text{g Pb/g}$ (SD= 9.6) for Cohort 3, reflecting declines in environmental lead levels over time; but did not differ appreciably by gender (10.2 $\mu\text{g Pb/g}$ (SD= 11.9) for males, 10.7 $\mu\text{g Pb/g}$ (SD= 11.7) for females). Children's mean blood lead level across all ages was 3.9 $\mu\text{g/dL}$ (SD= 3.05). Thirty-one percent of children had a blood lead level that exceeded 10 $\mu\text{g/dL}$ at some point in the study; but this accounted for only 8% of all blood lead measures.

Table II-2 shows the associations between patella lead and other child and maternal characteristics. Older mothers had a higher bone lead burden compared to younger mothers ($\beta= 0.52$, $SD= 0.06$). Primiparous women had lower patella lead levels than multiparous women. Patella bone lead was significantly associated with repeated measures of child blood lead up to 48 months.

Figure II-1 depicts the adjusted weight trajectories of children exposed to 2 SD above (high exposed) and 2 SD below (low exposed) the maternal patella lead sample mean, stratified by gender. Females exposed to high lead levels had a reduced weight trajectory compared to females exposed to low lead levels (Figure II-1, Panel A). The association between patella lead and weight was not statistically significant until 19 months of age when the mean weight difference between those exposed to low lead compared to those exposed to high lead was -280 g (95% CI= -570 to -3). Between 19 months and 5 years of age, the association of maternal patella lead with female's weight became significantly larger (see Panel A, P -value for trend <0.1). Among males, the difference in weight trajectories was not noticeably different between those exposed to low and those exposed to high maternal patella lead levels (Figure II-1, Panel B). Males with corresponding high maternal patella lead exposure had a seemingly higher weight trajectory, though not significantly different from those exposed to low patella lead, an effect opposite to what was observed among females. For example, at 12 months of age, the average weight of males exposed to high patella lead was 84.9 g (95% CI= -176.6 to 346.5) higher than males exposed to low patella lead.

Table II-3 gives the unadjusted and adjusted associations between a 1 SD increase in patella lead and weight at various ages. After controlling for children's concurrent blood lead levels, the association between patella lead and weight described above persisted. The association of patella lead with weight of females was statistically significant at 15 months of age when a 1 SD increase in patella lead was associated with a -77.2 g decrease in weight (95% CI= -152.8 to -1.5). Among males, a 1 SD increase in patella lead was seemingly but not substantially associated with a decrease in 13.6 g by 48 months of age (95% CI= -97.9 to 70.8).

In adjusted models, tibia lead was not associated with decreases in weight over time among males or females (results not shown). After adjusting for concurrent blood lead levels, the association of tibia lead with weight of males or females remained the same.

Figure II-2 illustrates the partial residual plots for the effect of 1 SD increase in patella lead on weight at selected ages, adjusted for all covariates, which provide evidence that the association between patella lead and weight was linear at each age. When child blood lead was added to the model, the association remained linear at every age (results not shown).

We observed no change in the association between patella lead and weight over time among females or males when the outliers of patella lead in the analytic sample (approximately 4.5 SD above the mean), were included in the model (results not shown). Although the mean weight difference between those exposed to low levels of lead and

those exposed to high levels was not statistically different, graphically the trajectories appeared to be distinct for both females and males (as what was observed in the original analytic sample among females, i.e. 2 curves not overlapping).

Discussion

This is the first longitudinal study to investigate the association of cumulative prenatal lead exposure as measured by maternal bone lead levels with child's weight trajectory between birth and 5 years of age. These results extend our previous findings (Gonzalez-Cossío, Peterson et al. 1997; Sanín, Gonzalez-Cossío et al. 2001), relating prenatal lead exposure with weight decrements at birth and 1-month of age, by examining associations with weight up to 5 years. Our results suggest that prenatal lead exposure is associated with a sustained decrease in child weight over time among females, but not males, independent of postnatal lead exposure and adjusting for several predictors of child's weight and other covariates.

Cross-sectional studies have investigated the association of child blood lead levels with weight (Ignasiak, Sławińska et al. 2006; Little, Spalding et al. 2009). Ignasiak et al. observed a 2800 g decrease in weight among males and 3500 g decrease among females for each 10 µg/dL increase in blood lead levels among 899 7 to 15 year-old youth in Poland (Ignasiak, Sławińska et al. 2006). Mean blood lead level in that study (7.7 µg/dL, SD= 3.5) however, was higher than in our study cohort (3.9 µg/dL, SD= 3.05). Little et al. found that a 10 µg/dL increase in concurrent child blood lead levels was associated with a 1900 g (95% CI= 1700 to 2100) decrease in weight among 360 children 2-12 years

of age in Dallas, Texas (Little, Spalding et al. 2009). Their study population consisted of 2 cohorts of children recruited 20 years apart. Mean blood lead of the first cohort was 24.8 $\mu\text{g}/\text{dL}$ (SD= 11.0) and of the second cohort was 1.8 $\mu\text{g}/\text{dL}$ (SD= 1.8) (Little, Spalding et al. 2009). In another longitudinal study, Schell et al. observed a decrease in weight-for-age z-score at 6 (-0.77, SE=0.34) but not 12 months of age associated with maternal second trimester blood lead level higher than the median ($\geq 3 \mu\text{g}/\text{dL}$) (Schell, Denham et al. 2009).

Fetal lead exposure may exert its effects on growth through effects on bone mass (which is related to weight). Campbell and colleagues observed that children with high blood lead levels ($\geq 15 \mu\text{g}/\text{dL}$) had higher bone densities than those with low blood lead levels ($<15 \mu\text{g}/\text{dL}$) - suggesting that lead may inhibit proteins involved in the growth of chondrocytes, thereby causing premature maturation of the chondrocytes, which may have resulted in higher bone densities among lead-exposed children (Campbell, Rosier et al. 2004). In our study, in contrast, we found that higher lead exposure was associated with decreased weight. Fetal lead exposure may also exert its effect on growth through disruption of thyroid signaling. Specifically, a lead-induced deficiency in maternal thyroid hormone (hypothyroidism) might limit the amount of thyroid hormone available to the fetus (Zoeller, Dowling et al. 2002) thus decreasing soft tissue and organ growth (Glinioer 1997; Hernández-Avila, Peterson et al. 2002). An epidemiological study in Kosovo found that maternal blood lead was negatively associated with maternal free thyroxine (FT4) among mothers with high blood lead levels (mean= 20.56 $\mu\text{g}/\text{dL}$, SD= 7.38) but not among those with lower blood lead levels (mean= 5.60 $\mu\text{g}/\text{dL}$; SD= 1.99). The authors also found an association between prenatal exposure to maternal FT4 and a

0.29 kg/m² increase in the rate of change of BMI between birth and 1 year of age (95% CI= 0.11 to 0.48) (Lamb, Janevic et al. 2008).

We did not find an association of tibia lead with weight of males or females. This might be due to the fact that lead accumulates slower in the cortical bone (tibia) than in the trabecular bone (patella). Trabecular bone is more vascularized, and thus reflects more recent lead exposure than cortical bone (Hu, Milder et al. 1989; Hu, Rabinowitz et al. 1998).

In adjusted models, maternal tibia lead was not associated with decreases in weight over time among females (results not shown). Among males, a 1 SD increase in tibia was noticeably related to an 82.2 g increase in weight at 36 months (95% CI= 8.2 to 156.1) and thereon. After adjusting for concurrent blood lead levels, the effect of tibia lead on weight of males was not significant.

We found differences in associations by gender. Cross-sectional lead levels have been associated with decreases in weight and BMI among females but not males between 7 and 14 years of age (Ignasiak, Sławińska et al. 2006). That study's authors suggested that the BMI result might have occurred because lead was more strongly related to females' than males' height. Among children, height is moderately correlated with BMI (Freedman and Sherry 2009), unlike among adults. Lead might disrupt the endocrine system's functions differently among males and females. The effect of lead on growth might be mediated by its impact on estrogen metabolism. Estrogen stimulates growth hormone secretion, which in turn leads to increased levels of IGF-1 and body growth (Juul 2001; Leung, Johannsson et al. 2004). Prenatal lead exposure has been

demonstrated to decrease levels of estradiol (Dearth, Hiney et al. 2002; Iavicoli, Carelli et al. 2004) and IGF-1 (Ronis, Badger et al. 1998) in female rats and mice. The suppression of estrogen by lead might cause low levels of growth hormone, and IGF-1 thus reduced weight gain in females.

The decrease in bone lead levels in our later cohorts reveals the reduction in environmental lead exposure particularly due to phasing out of leaded gasoline in Mexico in the mid-1990s (Romieu, Palazuelos et al. 1994). Bone lead levels in this study were comparable to those of a cohort of 700 women in Los Angeles in the mid to late 1990s where mean calcaneus lead (a measure of trabecular bone lead) was 10.7 $\mu\text{g Pb/g}$ (SD= 11.9) and tibia bone lead was 8.0 $\mu\text{g Pb/g}$ (SD= 11.4) (Rothenberg, Kondrashov et al. 2002). Bone lead levels in our study were, however, lower compared to a cohort of residents in Southern Ontario, in which tibia lead levels among women ages 15 to 40 were around 5 $\mu\text{g Pb/g}$ (Roy, Gordon et al. 1997) and among a cohort of women who had recently delivered in Boston in the late 1980s (mean patella lead level was 5 $\mu\text{g Pb/g}$) (Hu, Hashimoto et al. 1996). Children's blood lead levels in our pooled analysis were higher than mean levels among 1 to 5 year olds in the U.S. between 1999 and 2008 (EPA 2009).

We found that primiparous mothers had lower patella lead levels than multiparous women. This association was likely confounded by maternal age. In fact, when including maternal age in the model, the association of maternal patella lead with parity became non-significant.

Our study has several potential limitations. We did not consider the potential effect of other environmental pollutants on growth and their interaction with lead. Also, we did not account for adequacy of maternal or child dietary intake. However, despite these limitations, we believe our results are still valid given the longitudinal design of our study, the adjustment for several predictors and potential confounders, and the use of bone lead as a measure of cumulative prenatal lead exposure. Moreover, our results are generalizable to mother-children pairs as mothers in our study population comprised a large age range (15 to 44 years), and were from diverse SES (low to middle income).

Conclusion

Prenatal lead exposure reflected by maternal patella lead is associated with a lower weight trajectory among 0 to 5 year old female but not male offspring. These results indicate that the impact of fetal lead exposure on growth can persist for years, even after adjusting for postnatal lead exposure. Future research might investigate whether those effects persist into early adolescence.

Table II-1: Comparison of participants in combined cohort analysis and excluded participants

	Included ‡		N	Excluded		
	Mean or %	SD		Mean or %	SD	
Child Characteristics						
Male (%)	52.3		502	48.4		
Gestational age (months)	39.2	1.1	429	38.0	2.1	**
Cohort ¶			504			**
1	29.0			26.8		
2A	15.0			20.4		
2B	30.0			26.4		
3	26.0			26.4		
Birth weight (kg)	3.2	0.4	432	3.0	0.6	**
Maternal Characteristics						
Age at delivery (years)	25.7	5.3	436	25.6	5.4	
Calf circumference (cm)	34.1	3.0	361	34.4	3.8	
Height (cm)	154.6	5.7	421	154.0	6.1	
Marital status			433			**
Married	70.7			68.4		
With partner	21.8			19.6		
Single, separated, or divorced	7.5			12.0		
Education (years)	10.5	3.2	437	10.2	3.1	
Parity			438			
Primiparous	38.7			40.0		
1 previous child	34.4			34.0		
2+ previous children	26.9			26.0		
Smoked during pregnancy (%)	4.5		434	3.7		
Breastfed for 6 months (%)	68.1		501	61.3		**
Lead biomarkers						
Child blood lead (µg/dL)	3.8	2.9	347	3.8	3.0	
Patella (µg Pb/g)	10.4	11.8	258	12.9	15.9	**
Tibia (µg Pb/g)	8.7	9.7	254	10.2	11.8	**

Significance level: ** <0.05

‡ N=1000 except for tibia (N=846), and children's mean blood lead level across all ages (N=673).

¶ The sample population consists of the combination of three sequentially-enrolled longitudinal pooled birth cohorts

Table II-2: Associations between patella bone lead and child and maternal characteristics

	Effect estimate	SD
Child characteristics		
Gender		
Males	Referent	
Females	0.49	0.75
Gestational age (weeks)	0.04	0.34
Cohort		
1	Referent	
2A	-0.74	1.16
2B	-4.97	0.95 **
3	-6.44	0.98 **
Maternal characteristics		
Age at delivery (years)	0.52	0.06 **
Calf circumference (cm)	-0.03	0.12
Height (cm)	-0.11	0.06
Marital status		
Married	Referent	
With partner	-0.5	0.91
Single, separated, or divorced	-2.79	1.43
Education (years)	0.01	0.11
Parity		
Primiparous	Referent	
1 previous child	2.58	0.87 **
2+ previous children	4.32	0.93 **
Breastfed for 6 months	0.53	0.80
Calcium treatment during lactation	4.56	1.05 **
Calcium treatment during pregnancy	-5.1	1.08 **
Lead biomarkers		
Repeated measures of child blood lead ($\mu\text{g/dL}$) ‡		
12 months	0.50	0.12 **
24 months	0.39	0.10 **
36 months	0.39	0.12 **
48 months	0.54	0.14 **
60 months	0.29	0.22
Tibia ($\mu\text{g Pb/g}$)	0.42	0.03 **

Significance: ** <0.05

‡ Here we report the yearly blood lead measures at visits common to all 3 cohorts (except cohort 1 when last study visit was 48 months).

Table II-3: Associations between 1 standard deviation increase in maternal patella lead and weight of females and males at different ages

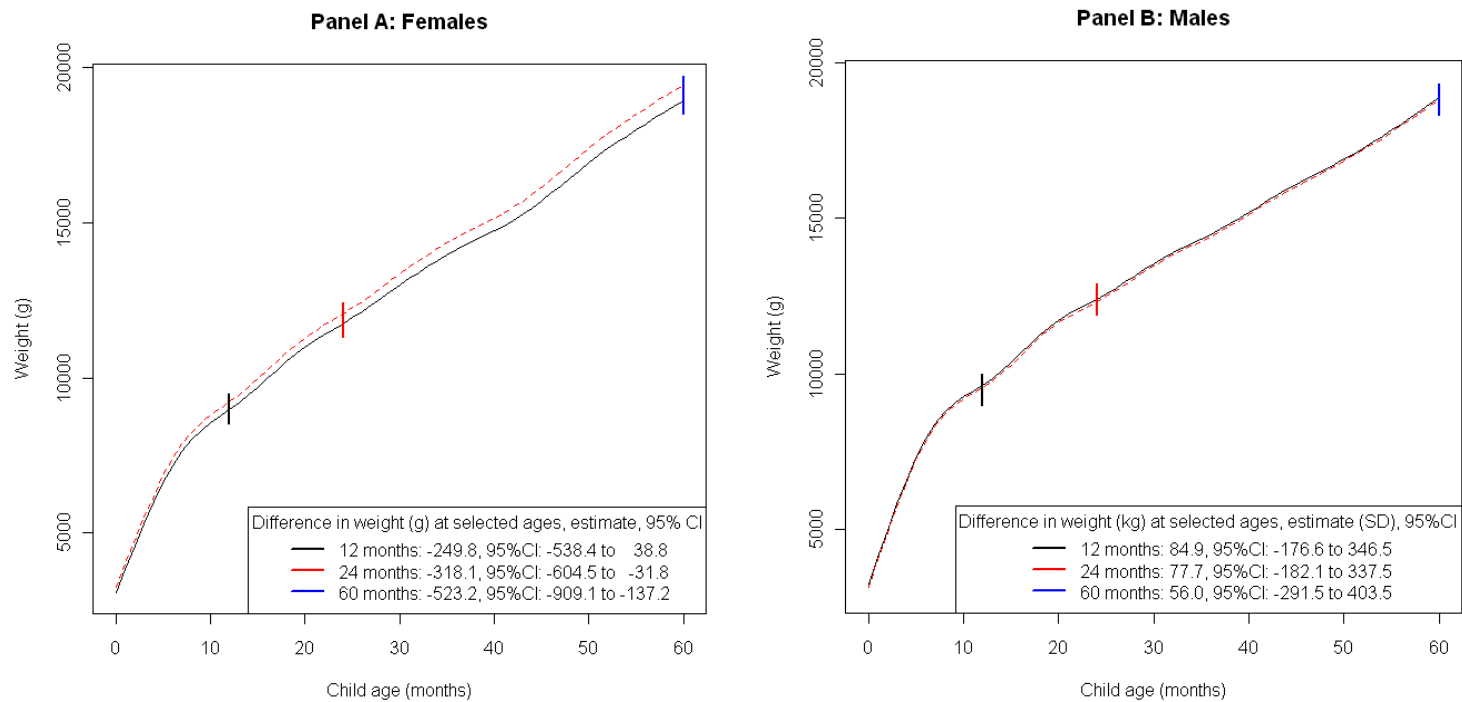
Age (months)	Unadjusted			Adjusted ‡			Fully adjusted ¶		
	β	95%CI		β	95%CI		β	95%CI	
Females									
Birth	-45.2	-145.6	55.2	-45.2	-122.8	32.3	-45.7	-131.7	40.2
12	-57.5	-152.8	37.7	-62.4	-134.5	9.7	-70.9	-147.9	6.0
24	-69.9	-164.3	24.6	-79.5	-151.1	-8.0	-96.1	-170.4	-21.8
36	-82.2	-180.4	16.0	-96.6	-172.6	-20.6	-121.3	-200.0	-42.6
48	-94.5	-200.4	11.4	-113.8	-198.5	-29.1	-146.4	-235.5	-57.4
60	-106.8	-223.6	10.0	-130.9	-227.4	-34.4	-171.6	-275.2	-68.0
Males									
Birth	27.2	-60.1	114.5	22.5	-47.5	92.6	72.3	-9.8	154.4
12	16.2	-66.4	98.8	20.6	-44.7	86.1	29.4	-42.1	100.8
24	5.2	-76.9	87.2	18.7	-46.2	83.7	27.8	-43.5	99.1
36	-5.8	-91.6	80.0	16.9	-52.0	85.8	7.9	-67.3	83.1
48	-16.8	-110.1	76.5	14.9	-61.5	91.5	-13.6	-97.9	70.8
60	-27.8	-131.6	76.0	13.0	-73.7	99.9	-35.0	-132.4	62.3

The effect estimates represent the association of 1 standard deviation increase in maternal patella lead ($\mu\text{g Pb/g}$) with weight (g) of males and females at different ages.

‡ Adjusted for cohort, maternal age at delivery, calf circumference, height, education, number of pregnancies, breastfeeding for 6 months, child's gestational age at birth and height.

¶ Additionally adjusted for repeated measures of concurrent child blood lead.

Figure II-1: Adjusted weight trajectory of children exposed to 2 standard deviations of patella lead above and below the mean

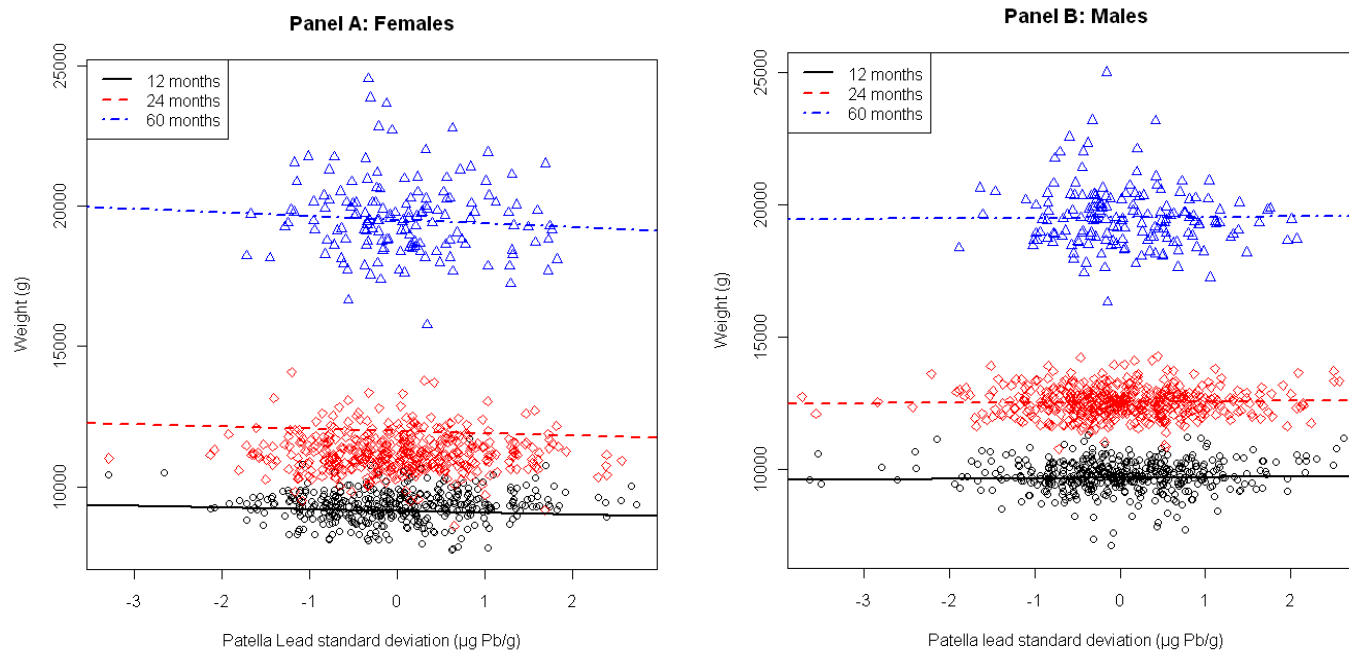


The dashed curve represents the average weight growth of children exposed to 2 standard deviations of maternal patella lead below the mean. The straight curve represents the average weight growth of children exposed to 2 standard deviations of maternal patella lead above the mean.

The weight trajectories are adjusted for cohort, maternal age at delivery, calf circumference, height, education, number of pregnancies, breastfeeding for 6 months, and child's gestational age at birth and height.

P-value for testing the difference of associations across age among females <0.1.

Figure II-2: Weight-patella association at various ages



These figures represent partial residual plots of weight and patella lead at each age. The different symbols reflect the data points at each age: triangles correspond to 60 months, kites to 24 months, and circles to 12 months.

For females, 1 standard deviation increase in maternal patella lead was associated with a weight decrease of 62.4 g (SD= 36.8) at 12 months; 79.5 g (SD= 36.5) at 24 months; 113.8 g (SD= 43.2) at 48 months; and 130.9 g (SD= 49.2) at 60 months. This decrease was statistically significant at 19 months.

For males, 1 standard deviation increase in maternal patella lead was associated with a weight increase of 20.6 g at 12 months; however this increase was not statistically significant.

References

- Andrews, K. W., D. A. Savitz, et al. (1994). "Prenatal lead exposure in relation to gestational age and birth weight: a review of epidemiologic studies." American Journal of Industrial Medicine **26**(1): 13-32.
- Aro, A. C., A. C. Todd, et al. (1994). "Improvements in the calibration of ¹⁰⁹Cd K x-ray fluorescence systems for measuring bone lead in vivo." Physics in Medicine and Biology **39**(12): 2263-71.
- Balleg, C., L. K. Khan, et al. (1999). "Blood lead concentration and children's anthropometric dimensions in the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994." The Journal of Pediatrics **134**(5): 623-30.
- Barker, D. J. (2006). "Adult consequences of fetal growth restriction." Clinical Obstetrics and Gynecology **49**(2): 270-83.
- Binkin, N. J., R. Yip, et al. (1988). "Birth weight and childhood growth." Pediatrics **82**(6): 828-34.
- Burger, D. E., F. L. Milder, et al. (1990). "Automated bone lead analysis by K-x-ray fluorescence for the clinical environment." Basic Life Sciences **55**: 287-92.
- Campbell, J. R., R. N. Rosier, et al. (2004). "The association between environmental lead exposure and bone density in children." Environmental Health Perspectives **112**(11): 1200-3.
- Cogswell, M. E. and R. Yip (1995). "The influence of fetal and maternal factors on the distribution of birthweight." Seminars in Perinatology **19**(3): 222-240.
- de Onis, M., C. Garza, et al. (2007). "Comparison of the WHO child growth standards and the CDC 2000 growth charts." The Journal of Nutrition **137**(1): 144-148.
- de Onis, M., A. Onyango, et al. (2006). "Comparison of the World Health Organization (WHO) Child Growth Standards and the National Center for Health Statistics/WHO international growth reference: implications for child health programmes." Public Health Nutrition **9**(7): 942-947.
- Dearth, R. K., J. K. Hiney, et al. (2002). "Effects of lead (Pb) exposure during gestation and lactation on female pubertal development in the rat." Reproductive Toxicology **16**(4): 343-52.

Dewey, K. G., M. J. Heinig, et al. (1993). "Breast-fed infants are leaner than formula-fed infants at 1 y of age: the DARLING study." The American Journal of Clinical Nutrition **57**(2): 140-145.

EPA. (2009). "Blood lead level." Retrieved April 10, 2010, from <http://cfpub.epa.gov/eroe/index.cfm?fuseaction=detail.viewInd&ch=49&IShowInd=0&subtop=381&lv=list.listByChapter&r=216631>.

Eriksson, J. G., T. Forsén, et al. (1999). "Catch-up growth in childhood and death from coronary heart disease: longitudinal study." British Medical Journal **318**(7181): 427-431.

Ettinger, A. S., H. Lamadrid-Figueroa, et al. (2009). "Effect of calcium supplementation on blood lead levels in pregnancy: a randomized placebo-controlled trial." Environmental Health Perspectives **117**(1): 26-31.

Ettinger, A. S., M. M. Téllez-Rojo, et al. (2004). "Effect of breast milk lead on infant blood lead levels at 1 month of age." Environmental Health Perspectives **112**(14): 1381-5.

Ettinger, A. S., M. M. Téllez-Rojo, et al. (2004). "Levels of lead in breast milk and their relation to maternal blood and bone lead levels at one month postpartum." Environmental Health Perspectives **112**(8): 926-31.

Freedman, D. S. and B. Sherry (2009). "The validity of BMI as an indicator of body fatness and risk among children." Pediatrics **124 Suppl 1**: S23-34.

Frisancho, A. R. and A. S. Ryan (1991). "Decreased stature associated with moderate blood lead concentrations in Mexican-American children." The American Journal of Clinical Nutrition **54**(3): 516-9.

Garza, C. and M. De Onis (1999). "A new international growth reference for young children." The American Journal of Clinical Nutrition **70**(1): 169S-172S.

Glinioer, D. (1997). "Maternal and fetal impact of chronic iodine deficiency." Clinical Obstetrics and Gynecology **40**(1): 102-16.

Gonzalez-Cossío, T., K. E. Peterson, et al. (1997). "Decrease in birth weight in relation to maternal bone-lead burden." Pediatrics **100**(5): 856-62.

Gonzalez-Cossío, T., L. H. Sanín, et al. (1998). "[Length and weight at birth: the role of maternal nutrition]." Salud Pública de México **40**(2): 119-26.

- Gulson, B., K. Mizon, et al. (2006). "Changes in manganese and lead in the environment and young children associated with the introduction of methylcyclopentadienyl manganese tricarbonyl in gasoline--preliminary results." Environmental Research **100**(1): 100-114.
- Gulson, B. L., C. W. Jameson, et al. (1997). "Pregnancy increases mobilization of lead from maternal skeleton." The Journal of Laboratory and Clinical Medicine **130**(1): 51-62.
- Gulson, B. L., J. G. Pounds, et al. (1999). "Estimation of cumulative lead releases (lead flux) from the maternal skeleton during pregnancy and lactation." The Journal of Laboratory and Clinical Medicine **134**(6): 631-40.
- Hernández-Avila, M., T. Gonzalez-Cossío, et al. (1996). "Dietary and environmental determinants of blood and bone lead levels in lactating postpartum women living in Mexico City." Environmental Health Perspectives **104**(10): 1076-82.
- Hernández-Avila, M., K. E. Peterson, et al. (2002). "Effect of maternal bone lead on length and head circumference of newborns and 1-month-old infants." Archives of Environmental Health **57**(5): 482-8.
- Hu, H., A. Aro, et al. (1995). "Bone lead measured by X-ray fluorescence: epidemiologic methods." Environmental Health Perspectives **103 Suppl 1**: 105-10.
- Hu, H., D. Hashimoto, et al. (1996). "Levels of lead in blood and bone of women giving birth in a Boston hospital." Archives of Environmental Health **51**(1): 52-8.
- Hu, H., F. L. Milder, et al. (1989). "X-ray fluorescence: issues surrounding the application of a new tool for measuring burden of lead." Environmental Research **49**(2): 295-317.
- Hu, H., F. L. Milder, et al. (1991). "The use of K X-ray fluorescence for measuring lead burden in epidemiological studies: high and low lead burdens and measurement uncertainty." Environmental Health Perspectives **94**: 107-10.
- Hu, H., M. Rabinowitz, et al. (1998). "Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms." Environmental Health Perspectives **106**(1): 1-8.
- Hwang, Y.-H., Y. Ko, et al. (2004). "Transition of cord blood lead level, 1985-2002, in the Taipei area and its determinants after the cease of leaded gasoline use." Environmental Research **96**(3): 274-282.

Iavicoli, I., G. Carelli, et al. (2004). "Effects of low doses of dietary lead on puberty onset in female mice." Reproductive Toxicology **19**(1): 35-41.

Ignasiak, Z., T. Sławińska, et al. (2006). "Lead and growth status of school children living in the copper basin of south-western Poland: differential effects on bone growth." Annals of Human Biology **33**(4): 401-414.

Jelliffe-Pawłowski, L. L., S. Q. Miles, et al. (2006). "Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes." Journal of Perinatology **26**(3): 154-162.

Jones, R., D. Homa, et al. (2009). "Trends in blood lead levels and blood lead testing among US children aged 1 to 5 years, 1988-2004." Pediatrics **123**(3): e376-e385.

Juul, A. (2001). "The effects of oestrogens on linear bone growth." Human Reproduction Update **7**(3): 303-313.

Kafourou, A., G. Touloumi, et al. (1997). "Effects of lead on the somatic growth of children." Archives of Environmental Health **52**(5): 377-83.

Kramer, M. S. (1987). "Determinants of low birth weight: methodological assessment and meta-analysis." Bulletin of the World Health Organization **65**(5): 663-737.

Kramer, M. S., L. Seguin, et al. (2000). "Socio-economic disparities in pregnancy outcome: why do the poor fare so poorly?" Paediatric and Perinatal Epidemiology **14**(3): 194-210.

Lamb, M. R., T. Janevic, et al. (2008). "Environmental lead exposure, maternal thyroid function, and childhood growth." Environmental Research **106**(2): 195-202.

Leung, K.-C., G. Johannsson, et al. (2004). "Estrogen Regulation of Growth Hormone Action." Endocrine Reviews **25**(5): 693-721.

Little, B. B., S. Spalding, et al. (2009). "Blood lead levels and growth status among African-American and Hispanic children in Dallas, Texas--1980 and 2002: Dallas Lead Project II." Annals of Human Biology **36**(3): 331-341.

Peterson, K. E., M. Salganik, et al. (2004). "Effect of succimer on growth of preschool children with moderate blood lead levels." Environmental Health Perspectives **112**(2): 233-7.

- Romieu, I., E. Palazuelos, et al. (1994). "Sources of lead exposure in Mexico City." Environmental Health Perspectives **102**(4): 384-9.
- Rosner, B. (1983). "Percentage points for a generalized ESD many-outlier procedure." Technometrics **25**(2): 165-172.
- Rothenberg, S. J., V. Kondrashov, et al. (2002). "Increases in hypertension and blood pressure during pregnancy with increased bone lead levels." American Journal of Epidemiology **156**(12): 1079-87.
- Roy, M. M., C. L. Gordon, et al. (1997). "Further experience with bone lead content measurements in residents of southern Ontario." Applied Radiation and Isotopes **48**(3): 391-6.
- Sanín, L. H., T. Gonzalez-Cossío, et al. (2001). "Effect of maternal lead burden on infant weight and weight gain at one month of age among breastfed infants." Pediatrics **107**(5): 1016-23.
- Schell, L., M. Denham, et al. (2009). "Growth of infants' length, weight, head and arm circumferences in relation to low levels of blood lead measured serially." American Journal of Human Biology **21**(2): 180-187.
- Schell, L. M., M. V. Gallo, et al. (2006). "Effects of pollution on human growth and development: an introduction." Journal of physiological anthropology **25**(1): 103-12.
- Schwartz, J., C. Angle, et al. (1986). "Relationship between childhood blood lead levels and stature." Pediatrics **77**(3): 281-8.
- Shukla, R., R. L. Bornschein, et al. (1989). "Fetal and infant lead exposure: effects on growth in stature." Pediatrics **84**(4): 604-12.
- Shukla, R., K. N. Dietrich, et al. (1991). "Lead exposure and growth in the early preschool child: a follow-up report from the Cincinnati Lead Study." Pediatrics **88**(5): 886-92.
- Téllez-Rojo, M. M., M. Hernandez-Avila, et al. (2004). "Impact of bone lead and bone resorption on plasma and whole blood lead levels during pregnancy." American Journal of Epidemiology **160**(7): 668-78.
- Zoeller, T. R., A. L. Dowling, et al. (2002). "Thyroid hormone, brain development, and the environment." Environmental Health Perspectives **110 Suppl 3**: 355-61.

CHAPTER III

Early Life Lead Exposure and Attained Height and BMI at 4 Years

Abstract

Background:

Early-life environmental lead exposure has been shown to have long-term health impacts on growth and development, but relatively few studies have examined lead's specific effects on height and BMI during potentially susceptible periods. Some research suggests that lead has an adverse effect on height (shorter) and BMI (greater) that is both exposure- and time-dependent and that may be wholly or partially reversible. However no study has examined the longitudinal association of pre- and post-natal lead exposure during susceptible periods on height and BMI at ages later than 33 months.

Methods:

The sample population consists of longitudinal pooled birth cohorts recruited between 1994 and 2005 in Mexico City. Lead exposure history categories were constructed based on exposure during the prenatal period (measured by maternal patella lead assessed at 1-month postpartum using *in vivo* K-X-ray fluorescence), infancy (average child's blood lead between birth to 24 months), and childhood (average child's blood lead between 30 and 48 months). Linear regression models adjusted for important

covariates were used to study the effect of exposure history on height and BMI at 48 months.

Results:

Children with higher lead exposure during mother's pregnancy attained a mean height that was somewhat shorter at 48 months (-0.32 cm, 95% CI= -0.90 to 0.25) than children with lower levels, regardless of post-natal exposure and adjusting for covariates. Children with high blood lead levels during infancy (birth to 24 months) attained a mean height at 48 months that was significantly shorter and with a greater effect size (-1.00 cm, 95% CI= -1.59 to -0.40, significant) than children with lower levels. Children with high blood lead levels during early childhood (30 to 48 months), on the other hand, were somewhat taller than their counterparts (0.50 cm, 95% CI = -0.08 to 1.09). This result was not significant, however.

Conclusion:

Our study suggests that early life lead exposure has a negative impact on skeletal growth that remains evident at 48 months of age, with an exposure window of greatest sensitivity occurring in infancy (birth to 24 months).

Introduction

Early life influences on development have been shown to have long-term health outcomes (Barker 2004). One such environmental influence is lead. Several studies have related lead exposure to childhood deficits in height (Schwartz, Angle et al. 1986; Shukla, Bornschein et al. 1989; Little, Snell et al. 1990; Frisancho and Ryan 1991; Shukla, Dietrich et al. 1991; Ballew, Khan et al. 1999; Hernández-Avila, Peterson et al. 2002; Ignasiak, Sławińska et al. 2006; Lamb, Janevic et al. 2008; Little, Spalding et al. 2009) and body mass index (BMI) (Ignasiak, Sławińska et al. 2006; Little, Spalding et al. 2009; Schell, Denham et al. 2009). Other studies did not find an association between lead exposure and BMI (Ballew, Khan et al. 1999), while other studies observed a higher BMI among lead-exposed children (Kim, Hu et al. 1995; Lamb, Janevic et al. 2008).

The effect of environmental influences such as lead on growth is not constant over time, and would be better defined through an exposure-time framework identifying sensitive periods. Sensitive windows of physical development have been portrayed as periods where exposure to environmental influences most detrimentally affects development later on (Tanner 1990; Lemasters, Perreault et al. 2000; Ben-Shlomo and Kuh 2002). Sensitive windows of exposure have been described in the literature of lifecourse epidemiology and developmental origins of adult disease (Lemasters, Perreault et al. 2000; Ben-Shlomo and Kuh 2002; Barker 2004): prenatal (from conception to birth), infancy (between birth and 24 months), and early childhood (after 24 months). During sensitive time periods, the developing system is highly susceptible to environmental insults given that developmental changes are occurring rapidly due to

increased cellular proliferation (Ben-Shlomo and Kuh 2002; Morford, Henck et al. 2004; Louis, Cooney et al. 2008).

In terms of exposure, infants aged 0 to 24 months and children between 24 to 48 months have different gastrointestinal absorptions of lead, respiratory ventilation rate, and proximity to the ground (EPA 1997; Selevan, Kimmel et al. 2000). Thus, younger children may have an increased lead exposure and dose.

Epidemiologic studies have suggested that the effect of lead may be wholly or partially reversible: early high lead exposure has been associated with decrements in attained length at 15 months (Shukla, Bornschein et al. 1989) but attenuation of exposure at later ages resulted in catch-up growth 33 months (Shukla, Dietrich et al. 1991). Another study observed a catch-up growth at 12 months (Schell, Denham et al. 2009) following early high lead exposure.

These results, however, are limited in terms of ages followed and lead exposure measures. Maternal bone lead concentration is a better biomarker of the cumulative maternal lead burden than is maternal blood lead during pregnancy (Hu 1998).

In the present study, we investigated the association of prenatal, infancy, and childhood lead exposure – different sensitive time windows – on attained height and BMI at 48 months.

Methods:

Study population

Similar exclusion criteria were applied to the three cohorts (Gonzalez-Cossío, Peterson et al. 1997; Téllez-Rojo, Hernández-Avila et al. 2002; Hernández-Avila, Gonzalez-Cossío et al. 2003). Additional eligibility criteria were applied for this study. Out of 1253 participants who showed up at the 48 months visit, 157 did not have a height measure obtained, and 185 were excluded due to missing information on the following variables: child weight and length at birth, gender, gestational age; and mother's age at delivery, calf circumference, height, educational level, marital status, parity, breastfeeding duration, and patella lead. Extreme outliers of child (weight and length at birth; weight and stature at 48 months, N=25) and maternal anthropometry (height and calf circumference, N=5) were identified. Subjects with outliers (maternal patella bone lead, N=6; and log-transformed child blood lead, N=173), or missing (23) lead measures were excluded. Also excluded were low birth weight (<2500 g, N=61) and premature neonates (<37 weeks of gestation, N=48), in order to minimize bias in the results due to postnatal catch-up characteristic of these infants (Barker, Osmond et al. 2005). Participants could have been excluded for multiple reasons, thus the final sample size consisted of 756 participants with complete height and 752 BMI measures at 48 months and all other covariates.

The research protocol was approved by the Ethics and Research Committees of the partnering institutions including the National Institute of Public Health of Mexico, the Harvard School of Public Health, the Brigham and Women's Hospital, the University of Michigan School of Public Health, and the participating hospitals.

Lead measurements

Bone Lead Measurements

Maternal bone lead was assessed at approximately 1-month postpartum using *in vivo* K-X-ray fluorescence (K-XRF). Measures were taken at the mid-tibial shaft (cortical bone) and the patella (trabecular bone). The instrument and validation have been extensively described (Burger, Milder et al. 1990; Hu, Milder et al. 1991; Aro, Todd et al. 1994; Hu, Aro et al. 1995). Analyses were conducted for maternal patella lead due to a smaller sample size of tibia measurements.

Blood Lead Measurements

Child blood was collected in trace metal-free tubes (BD Vacutainer® #367734, Becton-Dickinson, Franklin Lakes, NJ) between birth and 4 years of age and lead concentrations were measured using a graphite furnace atomic absorption spectroscopy (GFAAS) (Perkin-Elmer 3000, Chelmsford, MA, USA) at the metals laboratory of the American British Cowdray Hospital in Mexico City. External blinded quality control samples were analyzed by the Maternal & Child Health Bureau (MCHB) and the Wisconsin State Laboratory of Hygiene (WSLH) Cooperative Blood Lead Proficiency

Testing Program (PBPTP), as described elsewhere (Gonzalez-Cossío, Peterson et al. 1997).

Lead exposure history

To address the question of windows of vulnerability we created eight lead exposure history categories based on three windows during development: prenatal lead exposure (maternal patella lead); infancy lead exposure (average blood lead between birth and 24 months of age); early childhood lead exposure (average blood lead between 30 and 48 months of age). At each period, a child was classified as having high or low lead exposure defined by the median lead level at each period. Denoting H and L as high and low during each period (for example HHH = high prenatal, high infancy, high childhood), we created the following exposure categories for height: group 1 (HHH, n=156), group 2 (HHL, n=60), group 3 (HLH, n=51), group 4 (HLL, n=111), group 5 (LHH, n=112), group 6 (LHL, n=52), group 7 (LLH, n=60), and group 8 (LLL, n=155). Due to a smaller sample size, the number of participants in each lead exposure category slightly differed between height and BMI measures at 48 months (see Table III-1 and Figure III-1B).

Statistical analyses

Hypotheses and Power analyses

Our primary hypotheses of interest were: a) Prenatal lead exposure detrimentally affects child height and BMI at 48 months regardless of postnatal lead exposure; i.e.

children in the combined categories 1 to 4 will have lower height and BMI than children in the combined categories 5 to 8. b) Similarly, children exposed to high lead levels during infancy (categories 1, 2, 5, and 6) will have lower attained height and BMI than children exposed to low levels (categories 3, 4, 7, and 8). c) Children exposed to high lead levels during early childhood (groups 1, 3, 5, and 7) will have lower attained height and BMI than children exposed to low levels (groups 2, 4, 6, and 8). We also selected secondary hypotheses to explore differences between exposure subgroups (Table III-2). The power to detect a 1 cm difference ranged from 73% to 97%; and that of a 0.5 kg/m² difference from 84% to 99% across hypotheses.

Descriptive and bivariate analyses

Distributions were examined prior to statistical analyses. Extreme outliers of maternal and child anthropometry and lead measures were identified using the generalized extreme studentized deviation (ESD) method (Rosner 1983). Differences between participants with complete information and participants excluded due to the additional study eligibility criteria were compared using T-test for continuous variables and chi-squared test for categorical variables.

Group contrasts

We estimated linear regression models with exposure history groups coded as indicator variables. All models were adjusted for maternal height and calf circumference, number of previous pregnancies, marital status, education level, breastfeeding for 6 months, cohort, calcium treatment during lactation and pregnancy, age at delivery, and

gestational age. Height models were adjusted for birth length and BMI models for birth weight. Covariates were chosen based on biological relevance. From the adjusted models, we employed contrasts to compare the groups of lead exposure categories depicted by the hypotheses above. Data were analyzed using SAS 9.2 (Cary, NC, SAS Institute Inc.).

Results

Socio-demographic and other characteristics of the sample population (N=756) are presented in Table III-1. Median maternal patella bone lead was 10.4 $\mu\text{g Pb/g}$ (SD= 11.6). Median infancy child blood lead was 5.3 $\mu\text{g/dL}$ (SD= 2.8); median blood lead during early childhood 6.3 $\mu\text{g/dL}$ (SD= 2.9) (Table III-1). Certain characteristics differed between included and excluded participants. These differences were mainly due to the additional eligibility criteria of excluding low birth weight and premature infants, and to the outliers of anthropometry and lead measures. Another difference noted was in maternal educational level.

Figures III-1A and III-1B show the adjusted attained height and BMI at 48 months, respectively, by lead history categories. Children with the highest attained height were in group LLH; the lowest attained height in group LHL. Children with the highest attained BMI were in groups HLH and LLH whereas children with the lowest BMI were in HHL and LLL.

Table III-2 shows the unadjusted and adjusted results of hypothesis testing using general linear models. Children exposed to high levels of lead during pregnancy were not

significantly shorter at 48 months ($\beta= 0.32$, 95% CI= -0.90 to 0.25) than children exposed to lower levels, regardless of postnatal exposure and adjusting for covariates. Children exposed to high levels of lead during infancy (birth to 24 months) were 1 cm (95% CI= -1.59 to -0.40) shorter at 48 months than children exposed to low levels in adjusted models. In contrast, children exposed to high levels of lead during early childhood (30 to 48 months) were not significantly shorter at 48 months than children exposed to low levels ($\beta= 0.50$, 95% CI= -0.08 to 1.09), regardless of prior lead exposure.

Our secondary hypotheses consisted of comparing pairs or combinations of groups based on the general hypothesis that higher lead exposure earlier would lead to growth deficits. We found that children with consistently high levels of lead exposure (group 1, HHH) were 0.98 cm (95% CI= -1.87 to -0.10) shorter than children with consistently low levels of lead exposure throughout preschool years (group 8, LLL) (Table III-2). Children in group 1 (HHH) were marginally significantly shorter by 0.64 cm (95% CI= -1.33 to 0.05) than all other children in our study population. Similarly, children in group 8 (LLL) were notably taller compared to all other children. Exposure to high levels of lead during infancy (children in groups 1, HHH and 2, HHL) was significantly associated with a 1.32 cm (95% CI= -2.12 to -0.52) decrease in attained height at 48 months compared to exposure to low levels of lead (children in groups 7, LLH and 8, LLL).

Results for attained BMI at 48 months were in general in the same direction as for height. However, none of the groups compared were notably different (Table III-2). For example, children exposed to high levels of lead prenatally had a non-significant smaller

attained BMI at 48 months compared with children exposed to low levels of lead prenatally (-0.03 kg/m^2 , 95% CI= -0.27 to 0.21).

Discussion

We found that children who had been exposed to high levels of lead in infancy were shorter at 48 months than children exposed to lower levels. This may reflect that bone growth in children is most sensitive to lead exposure during those periods of development. High prenatal lead levels were not associated with deficits in height as compared to low prenatal levels. Exposure to lead during early childhood was also not associated with a lower attained height at 48 months.

The results of this study are consistent with the literature, where a 2 cm decreased length at 15 months was attributable to a $10 \text{ } \mu\text{g/dL}$ increase in 3 to 15 months blood lead levels and a high maternal blood lead during pregnancy ($\geq 7 \text{ } \mu\text{g/dL}$) (Shukla, Bornschein et al. 1989). In a follow-up study, the effect of prenatal lead was supplanted by infancy lead levels: children with high early infancy lead levels followed by low early childhood lead levels (regardless of prenatal exposure levels), had the highest attained height at 33 months; providing evidence of catch-up growth (Shukla, Dietrich et al. 1991). In another study, Schell and colleagues found that infants exposed to high levels of lead in utero ($\geq 3 \text{ } \mu\text{g/dL}$) followed by high postnatal lead levels ($\geq 6 \text{ } \mu\text{g/dL}$) had lower attained length-for-age (not significantly different), weight, arm and head circumference-for-age at 12 months (significantly different) than infants in all other exposure groups (Schell, Denham et al. 2009). On the other hand, infants exposed to high levels in utero followed by low

postnatal lead levels had higher anthropometric indexes at 12 months than any other exposure group (not significant for length-for-age); for example a 0.6 higher weight-for-age z-score than infants exposed to continuous high levels of lead (Schell, Denham et al. 2009).

Depending on where children are on the reference growth chart, a 1 cm decrement in height at 48 months could have different implications. For example, as compared to boys on the 50th percentile-for-age, a 1 cm height decrement could place them approximately at the 40th percentile-for-age (WHO Multicentre Growth Reference Study Group, 2006). As compared to girls aged 48 months in the 25th percentile-for-age, a 1 cm decrement in height could correspond to approximately the 17th percentile, which may entail considerable public health implications (WHO Multicentre Growth Reference Study Group, 2006).

The highest attained height was observed among children in the LLH and not the LLL group as would have been expected. Similarly, the lowest attained height was found among the LHL group and not the HHH group. Given the detrimental impact of lead on growth, the average difference in height between the HHH and LLL groups of 0.98 cm seems modest. On the other hand, Shukla and colleagues (1991) found a similar result where the average difference in height between the two extreme exposure groups was 0.99 cm (although they defined slightly different lead exposure time windows) (Shukla, Dietrich et al. 1991). Differences in our study may also have been attenuated by the error associated with our methods for measuring height.

We did not observe any effect of lead exposure history on BMI at 48 months. It is not known whether lead exposure affects child adiposity. However, as mentioned above, lead has been inconsistently associated with BMI, with some studies reporting BMI deficits (Ignasiak, Sławińska et al. 2006; Little, Spalding et al. 2009), other reporting increases in BMI (Kim, Hu et al. 1995; Lamb, Janevic et al. 2008). However, these studies have been limited by their cross-sectional design and lack of chronic exposure biomarkers (blood lead vs. bone lead). To further investigate this issue, we conducted exploratory analyses. Using the WHO growth standards, we calculated the proportion of overweight (\geq 85th percentile and $<$ 95th percentile-for-age, 10.8%) and obese (\geq 95th percentile, 5.4%) children at 48 months in each lead history category. The highest proportion of overweight and obese children was found in group LHH; the lowest proportion in group HHL. Due to a small sample size, we did not have the power to detect if the lead exposure categories were associated with the proportion of overweight and obese (the power ranged between 0.05 and 0.12).

Although height and BMI measure different aspects of growth (WHO 1995) and the effect of lead might occur through different mechanisms, sensitive periods are likely to be the same for both outcomes given that the sensitive periods defined are wide in age range and that exposure and absorbed dose considerably change between those periods. For example, the gastrointestinal absorption of lead for infants aged 0 to 2 years is between 42 and 53%, but lower for children 2 to 6 years old (between 30 and 40%) (Selevan, Kimmel et al. 2000).

Lead is thought to affect bone growth by impairing bone activity (osteoblasts) and/or bone cartilage by influencing epiphyseal growth plate chondrocytes (Ignasiak, Sławińska et al. 2006). Fetal osteoblasts are bone forming cells involved in the synthesis of type I collagen as well as alkaline phosphatase. Animal studies have shown that lead impairs osteoblast function by reducing osteoblast activity. Klein and Wiren observed a “dose-dependent inhibition” of alkaline phosphatase (important for mineral deposition), an enzymatic marker for osteoblast activity among rats (Klein and Wiren 1993). Puzas and colleagues recognized that lead alters the process of bone elongation by affecting chondrocytes (Puzas, Sickel et al. 1992). In fact, lead alters the signaling pathway of chondrocytes by affecting growth factors essential for chondrocytes maturation and regulation (Zuscik, Pateder et al. 2002). Zuscik et al. observed that lead alters the normal effects of chondrocytes terminal differentiation by acting on the transforming growth factor-beta1 (TGF- β 1) and other signaling pathways in chondrocytes (Zuscik, Pateder et al. 2002).

An objective of this study was to disentangle the effect of lead at each period. The different lead measures, however, were partially correlated, which was not surprising. Maternal patella lead was somewhat correlated with infancy blood lead ($r= 0.19, P<0.05$) and with early childhood blood lead ($r= 0.16, P<0.05$). Infancy and early childhood blood lead were moderately correlated ($r= 0.50, P<0.05$). We were thus able to more definitively disentangle prenatal vs. postnatal lead exposure more than infancy vs. early childhood lead exposure. Still, the exposure measures were not prohibitively correlated.

A relatively high number of subjects were excluded from the analytical sample (Table III-1) mainly due to missing demographic, anthropometric, and child exposure data. We do not suspect that selection bias could have resulted in an artifactual result given that the sample of included and excluded participants differed mainly due to exclusion of outliers and that maternal characteristics were comparable. These results are representative to Mexican children of low-to-moderate income reproductive-aged mothers. An alternative explanation for the results obtained could be that children's blood lead levels peak around 24 months (Bellinger 2004). However, in our study, the mean blood lead level of children from birth to 24 months was lower than from 30 to 48 months.

The analytical approach employed has some limitations. This method of averaging exposure during a time window assumes that exposure is constant over this specific period. However, two children who have similar blood lead during a specific period have not necessarily been exposed to the same concentration of lead. For example, we take the case of two children with the same average blood lead of 7 $\mu\text{g}/\text{dL}$ between birth and 24 months. The first child with two blood lead measures of 4 $\mu\text{g}/\text{dL}$ at 6 months and 10 $\mu\text{g}/\text{dL}$ at 12 months. The second with constant blood lead of 7 $\mu\text{g}/\text{dL}$ at two visits. The first child who experiences the peak in blood lead might experience a different effect on growth than a child with a relatively high, yet constant exposure. While exposure changes during each time window, our method assumes it is constant. However, this method is able to disentangle the effect of lead at each period defined largely. More refined periods would mean an increased number of lead exposure history but a smaller

sample size in each group, which could limit inferences. Also, this method has been previously used in the literature (Shukla, Dietrich et al. 1991; Schell, Denham et al. 2009).

Alternative approaches for investigating the effects of time-changing exposure have been proposed. For example, several methods in the life course literature (social epidemiology) and occupational exposure literature, among others, have been described. Some of the methods include analysis of risk accumulation, pathways, and estimating latency effects (Richardson 2009). Hallqvist and colleagues (2004) provide a review of the first two, as well as the critical periods approach we employed. The first method, risk accumulation, considers that the total duration and intensity of specific exposures leads to accrued disease risk later in life (Hallqvist, Lynch et al. 2004). The pathway approach proposes that changes in exposure throughout the life course might be an important predictor of health (Hallqvist, Lynch et al. 2004). Latency estimation refers to calculating the time between when the exposure occurred and when the health effect is observed. These three methods are interrelated, as they each provide information about timing of vulnerability. We chose the critical periods approach as it is most well suited to answering the research question we posed.

Conclusion

Our study suggests that early life lead exposure has a negative impact on skeletal growth that remains evident at 48 months of age, with an exposure window of greatest

sensitivity occurring in infancy (birth to 24 months). Prenatal lead exposure is not associated with height at 48 months as originally hypothesized. In addition, none of the lead exposure windows is related to BMI.

Table III-1: Comparison of included and excluded participants

	Included ‡		Excluded		
	Mean or %	SD	N	Mean or %	SD
Child Characteristics					
Male (%)	52.0		495	48.5	
Gestational age (months)	39.2	1.1	471	38.4	2.1 **
Birth length (cm)	50.4	1.9	456	49.7	3.3 **
Birth weight (kg)	3.2	0.4	470	3.1	0.5 **
BMI at 48 months (kg/m ²)	15.9	1.5	340	16.2	2.9 **
Height at 48 months (cm)	100.9	3.8	340	100.7	5.1
Cohort			497		**
1	24.9			47.7	
2A	15.9			15.7	
2B	33.7			15.7	
3	25.5			20.9	
Maternal Characteristics					
Age at delivery (years)	25.7	5.3	472	25.3	5.3
Calf circumference (cm)	34.2	3.0	407	34.2	3.6
Height (cm)	154.5	5.5	456	153.8	6.3
Marital status			469		
Married	71.2			67.4	
With partner	20.6			23.5	
Single, separated, or divorced	8.2			9.2	
Education (years)	10.5	3.1	473	9.9	3.0 *
Parity			473		
Primiparous	39.0			37.2	
1 previous child	35.1			33.0	
2+ previous children	25.9			24.9	
Breastfed for 6 months (%)	68.3		497	67.0	
Lead biomarkers					
Mean Child blood lead (µg/dL) from birth to 24 months	5.3	2.8	473	5.6	3.2 **
Mean Child blood lead (µg/dL) from 30 to 48 months	6.3	2.9	379	6.8	3.9 **
Patella (µg Pb/g)	10.4	11.6	327	13.3	15.9 **
Tibia (µg Pb/g)	8.7	9.8	325	10.5	11.1 **

Significance level: ** <0.05

N= 756 except for BMI at 48 months N=752, and tibia N=633

Figure III-1A: Lead exposure history and attained height at 48 months

70

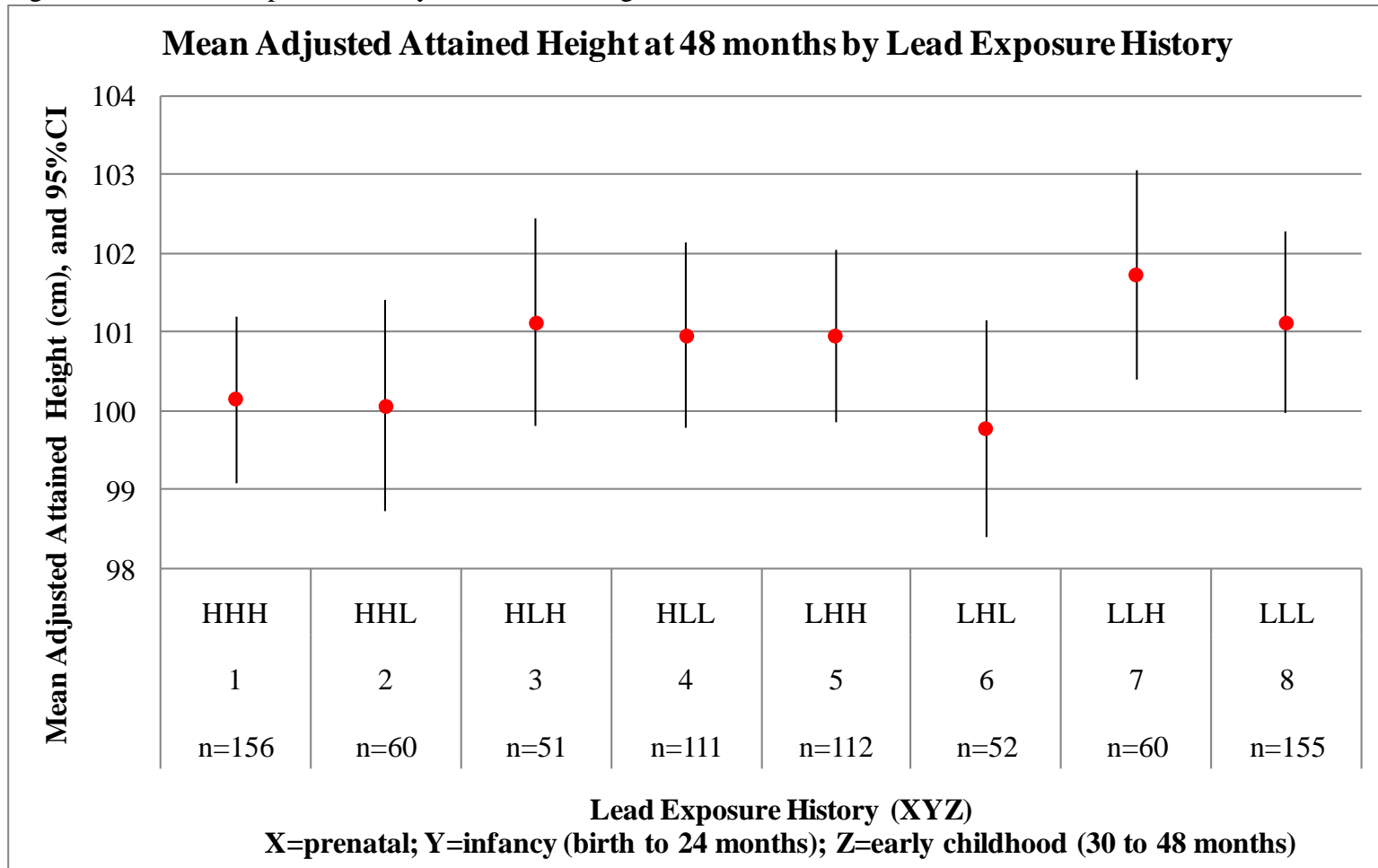


Figure III-1B: Lead exposure history and attained BMI at 48 months

71

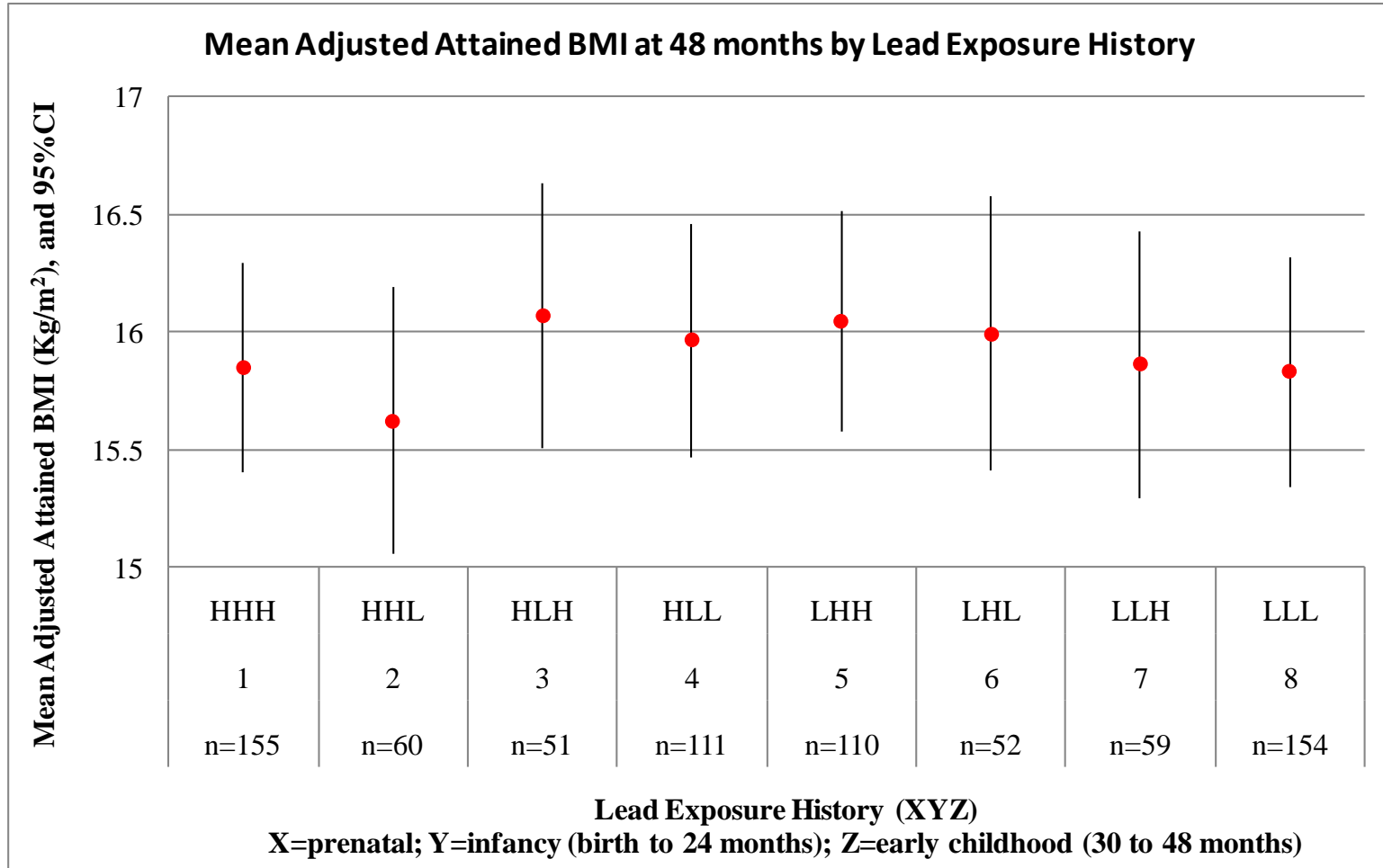


Table III-2: Hypotheses and estimates for height and BMI differences at 48 months

Hypothesis	Estimates for height differences (cm)				Estimates for BMI differences (kg/m ²)			
	Unadjusted		Adjusted ‡ †		Unadjusted		Adjusted ‡ ¶	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Primary hypotheses								
High vs. low prenatal	-0.37	-0.96 , 0.21	-0.32	-0.90 , 0.25	-0.08	-0.32 , 0.17	-0.03	-0.27 , 0.21
High vs. low infancy	-1.34	-1.93 , -0.76	-1.00	-1.59 , -0.40	-0.05	-0.29 , 0.19	-0.07	-0.32 , 0.18
High vs. low early childhood	0.47	-0.12 , 1.05	0.50	-0.08 , 1.09	0.08	-0.16 , 0.32	0.11	-0.14 , 0.35
Secondary Hypotheses								
Group HHH vs. LLL	-1.39	-2.22 , -0.57	-0.98	-1.87 , -0.10	-0.03	-0.37 , 0.31	0.03	-0.34 , 0.41
Group HHH vs. all others	-0.86	-1.53 , -0.19	-0.64	-1.33 , 0.05	-0.02	-0.29 , 0.26	-0.05	-0.34 , 0.24
Group LLL vs. all others	0.73	0.06 , 1.40	0.49	-0.18 , 1.16	0.02	-0.26 , 0.30	-0.09	-0.37 , 0.19
Groups HHH+HHL vs. LLH+LLL	-1.72	-2.50 , -0.93	-1.32	-2.12 , -0.52	-0.12	-0.45 , 0.20	-0.10	-0.44 , 0.23

N=756 for height models, and N=752 for BMI models

‡ Adjusted for maternal age at delivery, parity, marital status, education, breastfeeding, calcium treatment group, cohort, calf circumference, height, and child gender, and gestational age.

† Additionnally adjusted for child length at birth

¶ Additionnally adjusted for child weight at birth.

References

- Aro, A. C., A. C. Todd, et al. (1994). "Improvements in the calibration of ¹⁰⁹Cd K x-ray fluorescence systems for measuring bone lead in vivo." Physics in Medicine and Biology **39**(12): 2263-71.
- Balleg, C., L. K. Khan, et al. (1999). "Blood lead concentration and children's anthropometric dimensions in the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994." The Journal of Pediatrics **134**(5): 623-30.
- Barker, D. J. (2004). "The developmental origins of adult disease." Journal of the American College of Nutrition **23**(6 Suppl): 588S-595S.
- Barker, D. J. (2004). "Developmental origins of adult health and disease." Journal of Epidemiology and Community Health **58**(2): 114-5.
- Barker, D. J., C. Osmond, et al. (2005). "Trajectories of growth among children who have coronary events as adults." New England Journal of Medicine **353**(17): 1802-9.
- Bellinger, D. C. (2004). "Lead." Pediatrics **113**(4 Suppl): 1016-22.
- Ben-Shlomo, Y. and D. Kuh (2002). "A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives." International Journal of Epidemiology **31**(2): 285-293.
- Burger, D. E., F. L. Milder, et al. (1990). "Automated bone lead analysis by K-x-ray fluorescence for the clinical environment." Basic Life Sciences **55**: 287-92.
- EPA (1997). Exposure Factors Handbook (Final Report) U.S. Environmental Protection Agency, Washington, DC, EPA/600/P-95/002F a-c, 1997.
- Frisancho, A. R. and A. S. Ryan (1991). "Decreased stature associated with moderate blood lead concentrations in Mexican-American children." The American Journal of Clinical Nutrition **54**(3): 516-9.
- Gonzalez-Cossío, T., K. E. Peterson, et al. (1997). "Decrease in birth weight in relation to maternal bone-lead burden." Pediatrics **100**(5): 856-62.
- Hallqvist, J., J. Lynch, et al. (2004). "Can we disentangle life course processes of accumulation, critical period and social mobility? An analysis of disadvantaged socio-

economic positions and myocardial infarction in the Stockholm Heart Epidemiology Program." Social Science & Medicine **58**(8): 1555-1562.

Hernández-Avila, M., T. Gonzalez-Cossío, et al. (2003). "Dietary calcium supplements to lower blood lead levels in lactating women: a randomized placebo-controlled trial." Epidemiology **14**(2): 206-12.

Hernández-Avila, M., K. E. Peterson, et al. (2002). "Effect of maternal bone lead on length and head circumference of newborns and 1-month-old infants." Archives of Environmental Health **57**(5): 482-8.

Hu, H. (1998). "Bone lead as a new biologic marker of lead dose: recent findings and implications for public health." Environmental Health Perspectives **106 Suppl 4**: 961-7.

Hu, H., A. Aro, et al. (1995). "Bone lead measured by X-ray fluorescence: epidemiologic methods." Environmental Health Perspectives **103 Suppl 1**: 105-10.

Hu, H., F. L. Milder, et al. (1991). "The use of K X-ray fluorescence for measuring lead burden in epidemiological studies: high and low lead burdens and measurement uncertainty." Environmental Health Perspectives **94**: 107-10.

Ignasiak, Z., T. Sławińska, et al. (2006). "Lead and growth status of school children living in the copper basin of south-western Poland: differential effects on bone growth." Annals of Human Biology **33**(4): 401-414.

Kim, R., H. Hu, et al. (1995). "A longitudinal study of chronic lead exposure and physical growth in Boston children." Environmental Health Perspectives **103**(10): 952-7.

Klein, R. F. and K. M. Wiren (1993). "Regulation of osteoblastic gene expression by lead." Endocrinology **132**(6): 2531-2537.

Lamb, M. R., T. Janevic, et al. (2008). "Environmental lead exposure, maternal thyroid function, and childhood growth." Environmental Research **106**(2): 195-202.

Lemasters, G. K., S. D. Perreault, et al. (2000). "Workshop to Identify Critical Windows of Exposure for Children's Health: Reproductive Health in Children and Adolescents Work Group Summary." Environmental Health Perspectives **108**: 505-509.

Little, B. B., L. M. Snell, et al. (1990). "Blood lead levels and growth status of children." American Journal of Human Biology **2**(3): 265-269.

- Little, B. B., S. Spalding, et al. (2009). "Blood lead levels and growth status among African-American and Hispanic children in Dallas, Texas--1980 and 2002: Dallas Lead Project II." Annals of Human Biology **36**(3): 331-341.
- Louis, G. M. B., M. Cooney, et al. (2008). "Periconception window: advising the pregnancy-planning couple." Fertility and sterility **89**(2 Suppl): e119-e121.
- Morford, L., J. Henck, et al. (2004). "Hazard identification and predictability of children's health risk from animal data." Environmental Health Perspectives **112**(2): 266-271.
- Puzas, J. E., M. J. Sickel, et al. (1992). "Osteoblasts and chondrocytes are important target cells for the toxic effects of lead." Neurotoxicology **13**(4): 783-8.
- Richardson, D. B. (2009). "Latency models for analyses of protracted exposures." Epidemiology **20**(3): 395-399.
- Rosner, B. (1983). "Percentage points for a generalized ESD many-outlier procedure." Technometrics **25**(2): 165-172.
- Schell, L., M. Denham, et al. (2009). "Growth of infants' length, weight, head and arm circumferences in relation to low levels of blood lead measured serially." American Journal of Human Biology **21**(2): 180-187.
- Schwartz, J., C. Angle, et al. (1986). "Relationship between childhood blood lead levels and stature." Pediatrics **77**(3): 281-8.
- Selevan, S. G., C. A. Kimmel, et al. (2000). "Identifying critical windows of exposure for children's health." Environmental Health Perspectives **108 Suppl 3**: 451-5.
- Shukla, R., R. L. Bornschein, et al. (1989). "Fetal and infant lead exposure: effects on growth in stature." Pediatrics **84**(4): 604-12.
- Shukla, R., K. N. Dietrich, et al. (1991). "Lead exposure and growth in the early preschool child: a follow-up report from the Cincinnati Lead Study." Pediatrics **88**(5): 886-92.
- Tanner, J. M. (1990). Foetus into man: physical growth from conception to maturity. Cambridge, Massachusetts., Harvard University Press.
- Télliez-Rojo, M. M., M. Hernández-Avila, et al. (2002). "Impact of breastfeeding on the mobilization of lead from bone." American Journal of Epidemiology **155**(5): 420-8.

WHO (1995). Physical Status: The Use and Interpretation of Anthropometry. Report of a WHO Expert Committee, Geneva, Switzerland: World Health Organization.

WHO (Multicentre Growth Reference Study Group, 2006). WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development., Geneva: World Health Organization, 2006 (312 pages). .

Zuscik, M. J., D. B. Pateder, et al. (2002). "Lead alters parathyroid hormone-related peptide and transforming growth factor-beta1 effects and AP-1 and NF-kappaB signaling in chondrocytes." Journal of Orthopaedic Research **20**(4): 811-8.

CHAPTER IV

Environmental Lead Exposure and Puberty Timing

Abstract

Background:

Lead exposure during adolescence has been associated with delays in the onset of puberty. No study has examined the association of maternal bone lead as a marker of prenatal cumulative lead dose with puberty onset. This report describes the prospective epidemiological study design used to examine these associations and preliminary findings among in a subsample of 43 girls and 31 boys aged 6.5 to 15.5 years.

Methods:

Maternal bone lead was assessed at 1-month postpartum using *in vivo* K-X-ray fluorescence (K-XRF). Multivariable logistic regression was used to investigate the association of prenatal lead exposure with onset of puberty as assessed by self-reported Tanner stage greater or equal than 2 for genitalia (among boys), breast (among girls) and pubic hair development (for both genders), adjusting for child's age.

Results:

Among girls, mean age at study visit was 8.9 years (SD= 1.9); 38% were at stage B2 or above and 19% at stage P2 or above. Mean age at menarche was 10.3 years (SD=

1.5). Among boys, mean age was 9.7 years (SD= 2.5); 59% were at stage G2 or above, and 17% at stage P2 or above. For each 1 µg Pb/g increase in maternal patella lead, boys had a non statistically significant reduced odds of having entered stage P2 (OR= 0.94, 95% CI = 0.68, 1.30). The direction of associations was opposite for boys' genitalia, and for girls' breast and pubic hair development but also statistically non significant.

Conclusion:

These preliminary results suggest that prenatal lead exposure is not associated with pubertal onset in a longitudinal cohort study in Mexico, but interpretation is limited by small sample size. Future research in a larger sample from this cohort will further investigate the association of in utero lead exposure with onset of puberty.

Introduction

Environmental exposures may affect the timing and progression of puberty (Ouyang, Perry et al. 2005; Buck Louis, Gray et al. 2008; Euling, Selevan et al. 2008; Wolff, Britton et al. 2008). Alterations in the timing of puberty have been associated with important public health concerns such as modifications in physical growth of children (Ong, Northstone et al. 2007), metabolic (Frontini, Srinivasan et al. 2003) and cardiovascular diseases (Lakshman, Forouhi et al. 2009), and certain cancers (Golub, Collman et al. 2008; Dossus, Allen et al. 2010; Karageorgi, Hankinson et al. 2010) among adults.

Lead exposure in adolescence has been associated with delays in the timing of puberty in cross-sectional studies in nationally representative samples of U.S. females in NHANES III (Selevan, Rice et al. 2003; Wu, Buck et al. 2003; Gollenberg, Hediger et al. 2010). Selevan and colleagues (2003) found that concurrent blood lead levels were associated with significant delays in both breast (n=1986) and pubic hair (n=1964) development among African-American and Mexican-American but not among white girls. Among 1796 girls aged 8-16 years, blood lead levels of 3 µg/dL were associated with a 3.6-month delay in age at menarche compared with blood lead levels of 1 µg/dL; this finding however, was only observed among African-American girls (Selevan, Rice et al. 2003). Girls with blood lead levels between 2.1–4.9 µg/dL had a 52% reduced odds of having developed pubic hair and 58% reduced odds of having attained menarche as compared with girls with lower blood lead levels (0.7-2.0 µg/dL) (Wu, Buck et al. 2003). Among 138 Akwesasne Mohawk girls in New York State, lead levels above the

geometric mean (0.49 $\mu\text{g/dL}$) were correlated with a lower likelihood in attainment of menarche (Denham, Schell et al. 2005).

Among boys, evidence of delayed pubertal onset is sparser: just one study in Russia suggests a differential association of lead by pubertal characteristic: In 489 Russian boys aged 8-9 years, those with blood lead levels greater than or equal to 5 $\mu\text{g/dL}$ had a 44% reduced odds of having entered Tanner genital stage G2 (Hauser, Sergeyev et al. 2008). Blood lead levels were marginally significantly associated with decreased testicular volume but not with Tanner pubic hair stage P2 or higher (Hauser, Sergeyev et al. 2008). In the longitudinal follow-up of this cohort, baseline blood lead levels higher than 5 $\mu\text{g/dL}$ were related to an approximate 24% and 27% decrease, respectively, in the likelihood of pubertal onset as measured by G2 or higher and testicular volume greater than 3 mL. Blood lead levels were marginally significantly associated with a 31% reduction in the likelihood of pubertal onset measured by P2 or higher (Williams, Sergeyev et al. 2010).

This paper aims to address a research gap identified by an expert panel (Buck Louis, Gray et al. 2008) by examining the association of prenatal lead exposure with puberty onset. We report here on our prospective study design and preliminary findings using maternal bone lead as a marker of prenatal cumulative lead dose.

Methods

Study Population

The study population is derived from an on-going longitudinal birth cohort study in Mexico City. Study methods have been extensively described elsewhere (Gonzalez-Cossío, Peterson et al. 1997; Hernández-Avila, Peterson et al. 2002; Téllez-Rojo, Hernández-Avila et al. 2004; Ettinger, Lamadrid-Figueroa et al. 2009). Briefly, participants were recruited between 1994 and 2004 using similar methods and followed from birth to 5 years of age. Participants were then invited to participate in a later follow-up study in 2007. Exclusion criteria for the later follow-up study included having epilepsy or autism. All study participants completed a questionnaire on self-reported sexual maturation (described in detail below). Of the 95 children who filled out the questionnaire, 75 had a corresponding maternal patella bone lead measurement obtained at 1-month postpartum. One participant was excluded due to a biologically implausible value for BMI z-score. In this paper, we report preliminary results of 74 participants, which include 43 girls and 31 boys.

The study was approved by the Institutional Review Board at the University of Michigan and the Ethics Committee of the National Institute of Public Health in Mexico. The mother or guardian accompanying the child signed a consent form and the child provided his/her assent.

Self-Reported Sexual Maturation

We developed a questionnaire for self-report of sexual maturation based on adaptations to the original Tanner stages (Tanner 1962) with figures (obtained with permission from Taylor, Whincup et al. 2001; personal communication 9-29-08), and descriptions (obtained from Bonat, Pathomvanich et al. 2002; personal communication with senior author Margaret Keil 6-18-08) depicting the 5 Tanner stages. The questionnaire was translated into Spanish and reviewed by native speakers and field staff. It was first piloted among 12 participants aged 7 to 14 years old, and then given to the study population.

Self-reported sexual maturation has been extensively used in population studies (Jones, Hitchen et al. 2000; Rapkin, Tsao et al. 2006; Rubin, Maisonet et al. 2009) and specifically in Mexico (Posadas-Sánchez, Posadas-Romero et al. 2007; Denova-Gutiérrez, Jiménez-Aguilar et al. 2008). In population studies, self-reported sexual maturation has been correlated with physician-observed Tanner staging (Brooks-Gunn, Warren et al. 1987; Schlossberger, Turner et al. 1992). Some studies among obese adolescents' self-reported Tanner stages (Bonat, Pathomvanich et al. 2002; Lee, Valeria et al. 2006), but not others (Taylor, Whincup et al. 2001) found that fat tissue could be misinterpreted as breast tissue.

We used the self-reported sexual maturation figures from Taylor et al. (2001) because among boys the ratings for genital development are separate from pubic hair,

clearly distinguishing the effect of gonadal hormones (genital and breast development) and adrenal hormones (pubic hair). Through hypothalamic-pituitary-gonadal (HPG) axis hormone signaling the gonads are stimulated to produce androgen and estrogen which lead to the development of the penis, testes, breasts, ovaries, and uterus and also to the secondary sexual characteristics, such as pubic hair. Pubic hair development is also controlled by androstenedione and dehydroepiandrosterone (DHEA) released by the adrenal cortex in the hypothalamic-pituitary-adrenal (HPA) axis (Buck Louis, Gray et al. 2008).

Data Collection

The study visit included anthropometry and administration of self-reported sexual maturation questionnaire. The child psychologist explained the objective of the questionnaire to the mother and showed her a sample of the figures stating that they will ask their child if he/she would like to have them present in the room while he/she fills out the questionnaire. The psychologist then asked the mother if she would like to discuss the questionnaire with the child before he/she fills it out. The psychologist went through the questionnaire with the child and left him/her alone in the room. Children were asked to select their self-perceived stage of development by choosing the drawings and descriptions closest to their current stage of sexual development. Girls were asked to report their attainment of menarche. Both boys and girls reported any practices of pubic hair shaving as this might bias their self-reported Tanner staging. The questionnaire was

folded after completion. This allowed children confidentiality, privacy, and comfort with answering these sensitive questions.

Bone Lead Measurement

Maternal bone lead was assessed at 1-month postpartum using *in vivo* K-X-ray fluorescence (K-XRF). Measurements were obtained at the mid-tibial shaft (cortical bone) and the patella (trabecular bone) and reported in μg lead/g bone mineral. The instrument and validation have been extensively described (Burger, Milder et al. 1990; Hu, Milder et al. 1991; Aro, Todd et al. 1994; Hu, Aro et al. 1995).

Statistical analyses

Multivariable logistic regression was used to assess the association of prenatal lead exposure with puberty onset (Wu, Buck et al. 2003). Children were classified having entered puberty (dichotomous variable) if their self-reported Tanner stage was at or above 2. Our pubertal onset outcomes of interest were comprised of breast development greater than Tanner stage 2 for girls (denoted as B2), genitalia for boys (G2), and pubarche for both genders (P2).

Age-specific BMI and height z-scores were calculated using the SAS macro based on the 2007 World Health Organization (WHO) growth reference standards for 5-19 year olds (de Onis, Garza et al. 2007). Biologically implausible values were identified for height-for-age (defined as < -6 or > 5) and BMI-for-age (defined as < -5 or > 5) z-score.

Models were adjusted for child age at visit and maternal education (Wronka and Pawlińska-Chmara 2005). Given the limited sample size of children who had entered puberty, we could only include a maximum number of 1 predictor in the pubic hair regression models and 2 predictors in the breast and genitalia regression models (Peduzzi, Concato et al. 1996). Therefore, we adjusted for child's age in the pubic hair models; and for child's age and maternal education in the breast and genitalia development models. Because BMI has been related to pubertal onset in a number of studies (Kaplowitz, Slora et al. 2001; Wang 2002; Davison, Susman et al. 2003; Lee, Appugliese et al. 2007), we adjusted for BMI-for-age z-score in the second model in exploratory analyses. Analyses were conducted for patella lead due to a smaller sample size of tibia measurements. Data were analyzed using SAS 9.2 (Cary, NC, SAS Institute Inc.).

Results

Demographic and pubertal characteristics of 53 girls and 41 boys are shown in Table IV-1. Children were between 6.5 and 15.5 years old. Average patella lead was 11.2 $\mu\text{g Pb/g}$ (SD= 11.0) for girls, and 8.4 $\mu\text{g Pb/g}$ (SD= 8.7) for boys. Mothers had, on average, 11 years of education. Among girls, mean age was 8.9 (SD= 1.9) years; 38% were at stage B2 or above and 19% at stage P2 or above. Mean reported age at menarche among 6 girls was 10 years old. Among boys, mean age was 9.7 (SD= 2.5) years; 59% were at stage G2 or above and 17% at stage P2 or above. Most children reported not shaving their pubic hair (results not shown).

The logistic regression models depicted in Table IV-2 revealed that maternal patella lead was not associated with puberty onset. The direction of effect for maternal patella lead with boys' pubic hair was in the opposite direction than with boys' genitalia, girls' breast and pubic development. Adjusting for child's age, for each 1 $\mu\text{g Pb/g}$ increase in patella lead, boys had a 6% reduced odds of having entered stage P2 (95% CI= 0.68 to 1.30). After controlling for child's age at visit and maternal education, girls had a 5% increased odds of being in B2 for a 1 $\mu\text{g Pb/g}$ increase in maternal patella lead (95% CI= 0.97 to 1.13) (Table IV-2).

Child BMI was not associated with either maternal bone lead or puberty in this study. When including child BMI-for-age z-score in addition to child age and maternal education as predictors of puberty onset (for B2 and G2 outcomes), the effect estimates for patella lead were somewhat smaller, but not notably different than models without BMI-for-age z-score (results not shown).

Discussion

This is the first study to investigate the association of prenatal lead exposure, as measured by maternal bone lead, with pubertal onset as well as the first prospective study on lead's impact on puberty among both boys and girls. The small sample size available for this preliminary analysis limited inferences. Maternal patella lead was not related with an increased odds of delayed pubarche and breast development among girls and genitalia development among boys.

Maternal patella lead was not significantly associated with delayed pubertal onset as measured by stage P2 or greater, among boys. In the Chapaevsk, Russia cohort study, child blood lead levels ≥ 5 $\mu\text{g/dL}$ at 8-9 years among 461 boys were marginally significantly associated with a 31% reduction in the likelihood of pubertal onset in the longitudinal follow-up at 8 to 12 years of age measured by pubic hair staging 2 or higher (95% CI= 0.44 to 1.07) (Williams, Sergeyev et al. 2010). Child blood lead levels ≥ 5 $\mu\text{g/dL}$ at 8-9 years were also associated with a 24% and 27% decrease in the likelihood of pubertal onset at 8 to 12 years as measured by genitalia staging 2 or higher (95% CI= 0.59 to 0.98) and testicular volume greater than 3 mL (95% CI= 0.55 to 0.97), respectively (Williams, Sergeyev et al. 2010).

Mean age at menarche in our cohort (10.1, SD= 1.5) was younger than among 312 Mexican Americans 8 to 16 year old (12.2, 95% CI= 12.1 to 12.4) (Selevan, Rice et al. 2003). Age distribution of puberty onset was not comparable to U.S. nationally representative data of Mexican Americans in NHANES III (Sun, Schubert et al. 2002). Among girls, mean age at stages P2 and B2 were younger in our cohort (9.4, SD= 1.4 and 9.0, SD= 1.5, respectively) than among 763 Mexican Americans aged 7 to 19 (11.2, SE= 0.2 and 11.7, SE= 0.2, respectively). A similar observation was found among boys for stages P2 and G2 in our cohort (10.5, SD= 0.7 and 8.8, SD= 1.6, respectively) compared to Mexican Americans (12.2, SD= 0.2 and 11.1, SD= 0.2, respectively) (Sun, Schubert et al. 2002).

Lead has been associated with increases in BMI in certain epidemiologic studies (Kim, Hu et al. 1995; Lamb, Janevic et al. 2008). In Chapter III of this dissertation, we

found that maternal patella lead was not associated with child's BMI at 4 years of age. In this paper we found that maternal patella lead was still not a predictor of child BMI between 6.5 and 15.5 years of age. When including child BMI-for-age z-score as a predictor of B2 and G2, we found that the effect estimates were slightly reduced. However, child BMI-for-age z-score was not associated with any of the pubertal onset measures.

Rodent studies suggest that lead may have dual sites of action: at the level of hypothalamic pituitary unit and directly at the level of gonadal steroid biosynthesis (Buck Louis, Gray et al. 2008). Lead is believed to act on the hypothalamic-pituitary-gonadal axis (HPG) by blocking the release of gonadotropin-releasing hormone (GnRH), thus, decreasing puberty-related hormones such as insulin-like growth factor-1 (IGF-1) (Ronis, Badger et al. 1998), luteinizing hormone (LH), and estradiol (Dearth, Hiney et al. 2002). At the gonadal steroid biosynthesis level, lead has been shown to impair Leydig-cell and Sertoli cell functions (Ronis, Gandy et al. 1998; Gorbel, Boujelbene et al. 2002). A recent nationally-representative cross-sectional study of U.S. females found that lead was related to a decrease in serum inhibin B levels, but not with LH levels (Gollenberg, Hediger et al. 2010). This result is indicative of lead's direct action on the ovaries where inhibin B is produced (Gollenberg, Hediger et al. 2010).

These preliminary findings must be interpreted in light of several limitations. We relied on self-report for Tanner stage assessment which might introduce information bias. Others have shown that boys may overestimate their genitalia development

(Schlossberger, Turner et al. 1992; Lee, Valeria et al. 2006). We found that the directions were different for P2 and G2 in relation to maternal patella lead. In addition, we conducted analyses stratified by sex, so any misclassification for the outcome (puberty onset) introduced by sex would likely be non-differential and thus tend to inflate the standard error coefficients. The study sample was comprised of a small number of participants and, thus, may be underpowered to detect an association of lead exposure with pubertal development. In addition, due to the small sample, our ability to control for additional covariates of interest, such as child nutritional status and maternal age at menarche, was limited. Further study is needed using a prospective design with a larger sample size.

Conclusion

We found that maternal patella lead was not notably associated with pubertal onset among a small group of children followed prospectively since birth in Mexico City. However, given that these results are preliminary, we cannot rule out that prenatal lead exposure, as measured by maternal bone lead level, may be an important factor in pubertal onset. Since cumulative bone lead stores are long-lived, women and children may be exposed to lead even after environmental exposures have ceased. Future research will entail analyzing the association of prenatal lead exposure with onset of puberty among a larger sample of children from this cohort and include hormonal markers of pubertal development.

Table IV-1: Characteristics of the study population

	Girls (N=53)			Boys (N=41)		
	N	Mean or %	SD	N	Mean or %	SD
Demographic Characteristics						
Birth weight (kg)	51	3.0	0.4	38	3.2	0.6
Gestational age (months)	51	38.4	1.3	38	39.0	1.5
Child age at current visit (years)	53	8.9	1.9	41	9.7	2.5
Weight at current visit (kg)	53	32.3	9.8	41	38.1	15.1
Height at current visit (cm)	50	131.0	11.9	41	137.1	15.5
BMI-for-age z-score at current visit ¶	50	0.8	1.0	41	1.1	1.4
Height-for-age z-score at current visit ¶	50	0.0	0.8	41	0.2	0.8
Maternal education (years)	51	10.7	2.8	38	10.9	2.5
Lead Biomarkers						
Maternal Patella (µg Pb/g)	43	11.2	11.0	31	8.4	8.7
Maternal Tibia (µg Pb/g)	31	11.9	10.7	20	6.8	7.3
Tanner Stages						
Stage of breast development	53			41		
1	33	62.3		17	41.5	
2	17	32.1		13	31.7	
3	1	1.9		8	19.5	
4	2	3.8		3	7.3	
5	0	0.0		0	0.0	
Penis and scrotum development stage				41		
1				17	41.5	
2				13	31.7	
3				8	19.5	
4				3	7.3	
5				0	0.0	
Pubic hair stage	53			41		
1	43	81.1		34	82.9	
2	7	13.2		2	4.9	
3	2	3.8		4	9.8	
4	1	1.9		1	2.4	
5	0	0.0		0	0.0	

¶ Constructed using the SAS macro based on the 2007 WHO growth reference standards for 5-19 year olds

Table IV-2: Maternal patella lead and odds of puberty onset

	Adjusted model ‡ ^a			Fully adjusted model ‡ ^b		
	Odds			Odds		
	N	ratio	95% CI	N	ratio	95% CI
Girls						
Breast stage 2 or higher	43	1.04	0.97 , 1.11	43	1.05	0.97 , 1.13
Pubic hair stage 2 or higher	43	1.07	0.97 , 1.17			
Boys						
Genitalia stage 2 or higher	31	1.06	0.94 , 1.19	31	1.06	0.94 , 1.20
Pubic hair stage 2 or higher	31	0.94	0.68 , 1.30			

‡ Association between 1 µg Pb/g patella lead increase and odds of puberty entry

^a Adjusting for child's age at visit

^b Adjusting for child's age at visit, and maternal education

References

- Aro, A. C., A. C. Todd, et al. (1994). "Improvements in the calibration of ¹⁰⁹Cd K x-ray fluorescence systems for measuring bone lead in vivo." Physics in Medicine and Biology **39**(12): 2263-71.
- Bonat, S., A. Pathomvanich, et al. (2002). "Self-assessment of pubertal stage in overweight children." Pediatrics **110**(4): 743-747.
- Brooks-Gunn, J., M. P. Warren, et al. (1987). "Validity of self-report measures of girls' pubertal status." Child Development **58**(3): 829-41.
- Buck Louis, G. M., L. E. Gray, Jr., et al. (2008). "Environmental factors and puberty timing: expert panel research needs." Pediatrics **121 Suppl 3**: S192-207.
- Burger, D. E., F. L. Milder, et al. (1990). "Automated bone lead analysis by K-x-ray fluorescence for the clinical environment." Basic Life Sciences **55**: 287-92.
- Davison, K. K., E. J. Susman, et al. (2003). "Percent body fat at age 5 predicts earlier pubertal development among girls at age 9." Pediatrics **111**(4 Pt 1): 815-21.
- de Onis, M., C. Garza, et al. (2007). "Comparison of the WHO child growth standards and the CDC 2000 growth charts." The Journal of Nutrition **137**(1): 144-148.
- Dearth, R. K., J. K. Hiney, et al. (2002). "Effects of lead (Pb) exposure during gestation and lactation on female pubertal development in the rat." Reproductive Toxicology **16**(4): 343-52.
- Denham, M., L. M. Schell, et al. (2005). "Relationship of lead, mercury, mirex, dichlorodiphenyldichloroethylene, hexachlorobenzene, and polychlorinated biphenyls to timing of menarche among Akwesasne Mohawk girls." Pediatrics **115**(2): e127-34.
- Denova-Gutiérrez, E., A. Jiménez-Aguilar, et al. (2008). "Association between sweetened beverage consumption and body mass index, proportion of body fat and body fat distribution in Mexican adolescents." Annals of Nutrition & Metabolism **53**(3-4): 245-251.
- Dossus, L., N. Allen, et al. (2010). "Reproductive risk factors and endometrial cancer: the European Prospective Investigation into Cancer and Nutrition." International journal of cancer **127**(2): 442-451.

Ettinger, A. S., H. Lamadrid-Figueroa, et al. (2009). "Effect of calcium supplementation on blood lead levels in pregnancy: a randomized placebo-controlled trial." Environmental Health Perspectives **117**(1): 26-31.

Euling, S. Y., S. G. Selevan, et al. (2008). "Role of environmental factors in the timing of puberty." Pediatrics **121 Suppl 3**: S167-71.

Frontini, M. G., S. R. Srinivasan, et al. (2003). "Longitudinal changes in risk variables underlying metabolic Syndrome X from childhood to young adulthood in female subjects with a history of early menarche: the Bogalusa Heart Study." International Journal of Obesity **27**(11): 1398-1404.

Gollenberg, A. L., M. L. Hediger, et al. (2010). "Association Between Lead and Cadmium and Reproductive Hormones in Peripubertal U.S. Girls." Environmental Health Perspectives.

Golub, M. S., G. W. Collman, et al. (2008). "Public health implications of altered puberty timing." Pediatrics **121 Suppl 3**: S218-30.

Gonzalez-Cossío, T., K. E. Peterson, et al. (1997). "Decrease in birth weight in relation to maternal bone-lead burden." Pediatrics **100**(5): 856-62.

Gorbel, F., M. Boujelbene, et al. (2002). "[Cytotoxic effects of lead on the endocrine and exocrine sexual function of pubescent male and female rats. Demonstration of apoptotic activity]." Comptes Rendus Biologies **325**(9): 927-40.

Hauser, R., O. Sergeev, et al. (2008). "Association of blood lead levels with onset of puberty in Russian boys." Environmental Health Perspectives **116**(7): 976-980.

Hernández-Avila, M., K. E. Peterson, et al. (2002). "Effect of maternal bone lead on length and head circumference of newborns and 1-month-old infants." Archives of Environmental Health **57**(5): 482-8.

Hu, H., A. Aro, et al. (1995). "Bone lead measured by X-ray fluorescence: epidemiologic methods." Environmental Health Perspectives **103 Suppl 1**: 105-10.

Hu, H., F. L. Milder, et al. (1991). "The use of K X-ray fluorescence for measuring lead burden in epidemiological studies: high and low lead burdens and measurement uncertainty." Environmental Health Perspectives **94**: 107-10.

Jones, M. A., P. J. Hitchen, et al. (2000). "The importance of considering biological maturity when assessing physical fitness measures in girls and boys aged 10 to 16 years." Annals of Human Biology **27**(1): 57-65.

Kaplowitz, P. B., E. J. Slora, et al. (2001). "Earlier onset of puberty in girls: relation to increased body mass index and race." Pediatrics **108**(2): 347-53.

Karageorgi, S., S. E. Hankinson, et al. (2010). "Reproductive factors and postmenopausal hormone use in relation to endometrial cancer risk in the Nurses' Health Study cohort 1976-2004." International Journal of Cancer **126**(1): 208-16.

Kim, R., H. Hu, et al. (1995). "A longitudinal study of chronic lead exposure and physical growth in Boston children." Environmental Health Perspectives **103**(10): 952-7.

Lakshman, R., N. G. Forouhi, et al. (2009). "Early age at menarche associated with cardiovascular disease and mortality." The Journal of Clinical Endocrinology and Metabolism **94**(12): 4953-60.

Lamb, M. R., T. Janevic, et al. (2008). "Environmental lead exposure, maternal thyroid function, and childhood growth." Environmental Research **106**(2): 195-202.

Lee, J. M., D. Appugliese, et al. (2007). "Weight status in young girls and the onset of puberty." Pediatrics **119**(3): e624-30.

Lee, K., B. Valeria, et al. (2006). "Self-assessment of height, weight, and sexual maturation: validity in overweight children and adolescents." Journal of Adolescent Health **39**(3): 346-52.

Ong, K., K. Northstone, et al. (2007). "Earlier mother's age at menarche predicts rapid infancy growth and childhood obesity." PLoS medicine **4**(4): e132-e132.

Ouyang, F., M. J. Perry, et al. (2005). "Serum DDT, age at menarche, and abnormal menstrual cycle length." Occupational and Environmental Medicine **62**(12): 878-884.

Peduzzi, P., J. Concato, et al. (1996). "A simulation study of the number of events per variable in logistic regression analysis." Journal of Clinical Epidemiology **49**(12): 1373-1379.

Posadas-Sánchez, R., C. Posadas-Romero, et al. (2007). "Lipid and lipoprotein profiles and prevalence of dyslipidemia in Mexican adolescents." Metabolism, Clinical and Experimental **56**(12): 1666-1672.

- Rapkin, A. J., J. C. I. Tsao, et al. (2006). "Relationships among Self-Rated Tanner Staging, Hormones, and Psychosocial Factors in Healthy Female Adolescents." Journal of Pediatric and Adolescent Gynecology **19**(3): 181-187.
- Ronis, M. J., T. M. Badger, et al. (1998). "Endocrine mechanisms underlying the growth effects of developmental lead exposure in the rat." Journal of Toxicology and Environmental Health Part A **54**(2): 101-20.
- Ronis, M. J., J. Gandy, et al. (1998). "Endocrine mechanisms underlying reproductive toxicity in the developing rat chronically exposed to dietary lead." Journal of Toxicology and Environmental Health Part A **54**(2): 77-99.
- Rubin, C., M. Maisonet, et al. (2009). "Timing of maturation and predictors of menarche in girls enrolled in a contemporary British cohort." Paediatric and Perinatal Epidemiology **23**(5): 492-504.
- Schlossberger, N. M., R. A. Turner, et al. (1992). "Validity of self-report of pubertal maturation in early adolescents." Journal of Adolescent Health **13**(2): 109-13.
- Selevan, S. G., D. C. Rice, et al. (2003). "Blood lead concentration and delayed puberty in girls." New England Journal of Medicine **348**(16): 1527-36.
- Sun, S. S., C. M. Schubert, et al. (2002). "National Estimates of the Timing of Sexual Maturation and Racial Differences Among US Children." Pediatrics **110**(5): 911-919.
- Tanner, J. M. (1962). Growth at adolescence: with a general consideration of the effects of hereditary and environmental factors upon growth and maturation from birth to maturity. Oxford, Blackwell Scientific Publications.
- Taylor, S. J., P. H. Whincup, et al. (2001). "Performance of a new pubertal self-assessment questionnaire: a preliminary study." Paediatric and Perinatal Epidemiology **15**(1): 88-94.
- Téllez-Rojo, M. M., M. Hernández-Avila, et al. (2004). "Impact of bone lead and bone resorption on plasma and whole blood lead levels during pregnancy." American Journal of Epidemiology **160**(7): 668-78.
- Wang, Y. (2002). "Is obesity associated with early sexual maturation? A comparison of the association in American boys versus girls." Pediatrics **110**(5): 903-910.
- Williams, P., O. Sergeev, et al. (2010). "Blood lead levels and delayed onset of puberty in a longitudinal study of Russian boys." Pediatrics **125**(5): e1088-e1096.

Wolff, M. S., J. A. Britton, et al. (2008). "Environmental exposures and puberty in inner-city girls." Environmental Research **107**(3): 393-400.

Wronka, I. and R. Pawlińska-Chmara (2005). "Menarcheal age and socio-economic factors in Poland." Annals of Human Biology **32**(5): 630-8.

Wu, T., G. M. Buck, et al. (2003). "Blood lead levels and sexual maturation in U.S. girls: the Third National Health and Nutrition Examination Survey, 1988-1994." Environmental Health Perspectives **111**(5): 737-41.

CHAPTER V

Conclusion

Lead exposure at current environmental levels continues to pose a public health concern. One of our research goals was to evaluate the association of prenatal lead exposure measured by a novel biomarker, maternal bone lead, with physical growth and maturation. The objective of Chapter II was to expand on the limited number of longitudinal studies considering the association of prenatal lead exposure with children's weight from birth to school entry given that lead exposure has been more consistently associated with height than with weight. The aim of Chapter III was to disentangle the association of different windows of lead exposure susceptibility with height and BMI among children at 4 years old. Previous studies have investigated the longitudinal association of pre- and post-natal lead exposure during susceptible periods with height and BMI but only up until 33 months of age. In addition, only a limited number of studies have investigated the association of lead exposure with BMI, but none on childhood overweight or obesity. The goal of Chapter IV was to present preliminary results on the association of prenatal lead exposure with self-reported sexual maturation among a sample of the prospective cohort follow-up study.

The results of Chapter II fill a gap in the literature as they expanded on previous findings. Maternal bone lead burden during pregnancy had been associated with weight decrements up to 1 month of age. We found that maternal bone lead was associated with decrements in child weight from birth to 5 years of age. We noted that this association was observed only among females and not males, highlighting lead might have a differential effect on physical growth by gender.

Because variance in weight can be related to height or the relationship of weight for height (proportionality), we investigated in Chapter III the association of lead exposure with height and BMI. We were able to disentangle the effect of prenatal from postnatal lead exposure on attained height and BMI at 4 years of age providing evidence on windows of lead exposure susceptibility of child growth. We hypothesized that the most susceptible window would be the prenatal and infancy periods. Instead, we found that the infancy (birth to 24 months) blood lead levels were most influential on attained height at 4 years. An alternative explanation for the results obtained could be that children's blood lead levels have been found to peak around 24 months (Bellinger 2004). However, in our study, the mean blood lead level of children from birth to 24 months was lower than from 30 to 48 months.

Future research directions for Chapters II and III would need to investigate whether those associations persist in adolescence. Specifically, would the weight and height decrements observed continue at or beyond adolescence (i.e., would prenatal or

infancy lead exposure be associated with adult height – following the adolescent growth spurt)? Would a latent effect on BMI, which we did not observe, emerge at later ages?

In Chapter IV, we presented preliminary results of lead's association with puberty onset among a subset of 43 girls and 31 boys aged 6.5 to 15.5 years. We described our study design and the development of self-reported sexual maturation ratings identifying gender-specific secondary sexual characteristics that reflect different aspects of hormonal control of development. The results suggest that lead exposure was not associated with delayed pubertal onset of boys or girls (measured by pubic hair, genitalia, and breast development). A limitation of this study was the small sample size.

Future work will analyze the association of concurrent lead exposure (as measured by child blood lead) on puberty onset. Future research will involve administration of the self-reported questionnaire through continued follow-up in the longitudinal study. Further, a validation study will shed light on the reliability of the self-reported questionnaires as compared to physician ratings of Tanner staging.

How do these results relate to health policies regarding lead? Currently the CDC's blood lead level of concern is set at 10 $\mu\text{g}/\text{dL}$ (CDC 1991). It is not a threshold level or a health standard but, instead, a prompt for action to decrease child lead exposure by providing caregivers education, and triggering environmental investigation and follow-up testing (Committee on Environmental Health 2005; Advisory Committee on Childhood Lead Poisoning Prevention 2007). Blood lead levels less than 10 $\mu\text{g}/\text{dL}$ have been shown

to be associated with numerous adverse health effects (Canfield, Henderson et al. 2003; Téllez-Rojo, Bellinger et al. 2006; Surkan, Zhang et al. 2007). In Chapter III, child blood lead levels (mean= 5.3 $\mu\text{g/dL}$, SD= 2.8) was associated with decrements in attained height at 4 years of age. Because of the absence of a blood lead level threshold for health effect, the Agency for Toxic Substances and Disease Registry (ATSDR) has not set a minimum risk level (MRL) nor has the Environmental Protection Agency (EPA) put forth a reference dose (RfD) (ATSDR 2007). However, some researchers are advocating for a decrease in the CDC's blood lead level of concern from 10 $\mu\text{g/dL}$ to 5 $\mu\text{g/dL}$, which the CDC has so far declined to do (Bellinger 2008; CDC 2009). Our data supports this decrease. Others are arguing for a decrease to 2 $\mu\text{g/dL}$ (Gilbert and Weiss 2006). The proposal to decrease the CDC's level of concern from 10 $\mu\text{g/dL}$ to 5 $\mu\text{g/dL}$ or 2 $\mu\text{g/dL}$ levels has less to do with the biological significance of these levels than with the prevention of lead poisoning. There is no longer significant doubt in the majority of the scientific community that low-lead exposure as measured by child blood lead levels below 10 $\mu\text{g/dL}$ is associated with detrimental health outcomes. The translation of this information, however, is arguably constrained by political and policy considerations.

Currently, there are no health policies setting a level of concern for maternal bone lead concentrations. In Chapter II, we found that prenatal lead exposure, measured by maternal bone lead, was associated with decrements in weight. The publications from this research will hopefully contribute to the epidemiological evidence that maternal bone lead burden is associated with detrimental effects on offspring anthropometry. Efforts to develop recommendations related to maternal lead burden are ongoing, including the

imminent release of the long-awaited report of the CDC Committee on Lead and Pregnancy (CDC 2010).

The effect of lead on growth and development may be generalized to developing countries that have recently phased-out leaded gasoline (for example, the use of leaded gasoline was reduced in Lebanon in 2000). Given the long retention time of lead in the bones, the detrimental effects associated with lead exposure are far from being over. Other modern environmental health hazards (Nweke and Sanders III 2009) such as such as pesticides (dichlorodiphenyltrichloroethane or DDT) and polychlorinated biphenyls (PCBs), affect growth through endocrine disruption (Brucker-Davis, Wagner-Mahler et al. 2010; Schell and Gallo 2010). Emerging contaminants such as bisphenol A (BPA) and phthalates could potentially have consequences on reproductive health (Meeker, Hu et al. 2009). In addition, exposure to metal mixtures (or other contaminants such as manganese) and genetic susceptibilities may also play a role in growth and development. Understandably, not all possible factors can be accounted for in population studies. However, it is important to place this dissertation into perspective, and keep in mind that the goal of this dissertation was to shed light on the longitudinal effect of pre- and post-natal lead exposure. The results of this research are applicable to future policies on the use and development of new and old pollutants.

References

- Advisory Committee on Childhood Lead Poisoning Prevention, C. D. C. (2007). "Interpreting and Managing Blood Lead Levels <10 µg/dL in Children and Reducing Childhood Exposures to Lead." MMWR. Morbidity and Mortality Weekly Report **56**(RR08): 1-14.
- ATSDR (2007). Toxicological Profile for Lead. U.S. Department of Health and Human Services. Public Health Service. Atlanta, GA.
- Bellinger, D. C. (2004). "Lead." Pediatrics **113**(4 Suppl): 1016-22.
- Bellinger, D. C. (2008). "Very low lead exposures and children's neurodevelopment." Current Opinion in Pediatrics **20**(2): 172-177.
- Brucker-Davis, F., K. Wagner-Mahler, et al. (2010). "Exposure to selected endocrine disruptors and neonatal outcome of 86 healthy boys from Nice area (France)." Chemosphere **81**(2): 169-176.
- Canfield, R., C. Henderson, et al. (2003). "Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter." New England Journal of Medicine **348**(16): 1517-1526.
- CDC (1991). Preventing lead poisoning in young children. Atlanta, GA, US Department of Health and Human Services.
- CDC. (2009). "Why not change the blood lead level of concern at this time?" Retrieved October 14, 2010, from <http://www.cdc.gov/nceh/lead/policy/changeBLL.htm>.
- CDC. (2010). "Lead and Pregnancy Work Group Charge." Retrieved October 15, 2010, from <http://www.cdc.gov/nceh/lead/ACCLPP/pregnancyWG.htm>.
- Committee on Environmental Health (2005). "Lead Exposure in Children: Prevention, Detection, and Management." Pediatrics **116**(4): 1036-1046.
- Gilbert, S. G. and B. Weiss (2006). "A rationale for lowering the blood lead action level from 10 to 2 [µ]g/dL." Neurotoxicology **27**(5): 693-701.
- Meeker, J., H. Hu, et al. (2009). "Urinary phthalate metabolites in relation to preterm birth in Mexico city." Environmental Health Perspectives **117**(10): 1587-1592.

Nweke, O. C. and W. H. Sanders III (2009). "Modern Environmental Health Hazards: A Public Health Issue of Increasing Significance in Africa." Environmental Health Perspectives **117**(6).

Schell, L. M. and M. V. Gallo (2010). "Relationships of putative endocrine disruptors to human sexual maturation and thyroid activity in youth." Physiology & Behavior **99**(2): 246-253.

Surkan, P. J., A. Zhang, et al. (2007). "Neuropsychological function in children with blood lead levels <10 microg/dL." Neurotoxicology **28**(6): 1170-7.

Télliez-Rojo, M. M., D. C. Bellinger, et al. (2006). "Longitudinal associations between blood lead concentrations lower than 10 microg/dL and neurobehavioral development in environmentally exposed children in Mexico City." Pediatrics **118**(2): e323-30.