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Decrease in Bone Mineral Density during Pregnancy and Change in Maternal Blood Lead Levels

By

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Master of Public Health

Epidemiology

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By

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B.S., University of California, Davis, 2009

Thesis Committee Chair: Penelope P. Howards, Ph.D.

An abstract of
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Abstract

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By Heejoo Jo

Prenatal exposure to lead has been associated with spontaneous abortions, low birth weight, and developmental delay. Lead stored in women's bones is released into their blood during pregnancy. Maternal blood lead can cross the placenta and expose the fetus. The purpose of this study is to examine the relation between change in bone mineral density (BMD) and change in maternal blood lead levels across pregnancy. Additionally, this study evaluated whether the association between changes in BMD and blood lead is confounded by self-reported daily calcium intake. This was a prospective study of 100 women who received prenatal care during the first 12 weeks of pregnancy at Magee-Women's Hospital in Pittsburgh, Pennsylvania from October 1992 to February 1995. Change in maternal blood lead was calculated using the difference between blood lead levels (μg/dL) measured, on average, at 17 and 37 weeks gestation. Change in BMD was calculated using the difference between measurements of the ultra-distal BMD at 16 and 36 weeks gestation. Information regarding sociodemographic and lifestyle factors was collected during interviews between 8 and 18 weeks gestation and again between 31 and 41 weeks gestation. As expected, maternal blood lead levels increased and ultra-distal BMD decreased during pregnancy. However, change in BMD was weakly associated with change in maternal blood lead levels during pregnancy (β = 0.33 μg/dL, 95% CI = -8.45, 7.80) in a linear regression model adjusted for age, race, smoking, BMI, and calcium intake. Change in blood lead was higher among women whose calcium intake was <1,000 mg/day compared to 1,000-<2,000 mg/day. These findings suggest that the observed increase in maternal blood lead during pregnancy may not be predominantly due to the increased mobilization of bone lead as previously hypothesized. Further research on the mechanisms that contribute to increased maternal blood lead during pregnancy is needed.

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CHAPTER I: BACKGROUND

I. Introduction

A leading public health concern is the adverse effects from environmental exposure of lead on women and children. The effects of exposure to lead have been studied extensively in the past and continue to be studied today due to the persistence of lead in the environment. Prenatal exposure to lead is associated with spontaneous abortions in addition to lower birth weights and smaller head circumferences among infants. Additionally, children with increased blood lead levels have displayed developmental delays and lower intelligence (1). Mean blood lead levels higher than the national average of 0.14 μmol/L (2.8 μg/dL) still persist in subpopulations of children aged 1 to 5 years especially those of lower socioeconomic status (2). Currently, children are exposed to lead in-utero as well as in the postnatal environment. External sources of lead for both women and children include lead-based paint used in houses built before 1978, lead-contaminated household dust, soil, drinking water from corroded pipes or faucets, and imported goods such as children's toys or pottery. Women can also be occupationally exposed to lead from jobs involving construction, plumbing, or recycling. Lead from these sources are stored in the women's bones and released into their blood during pregnancy. Lead in maternal blood can cross the placenta and expose the fetus. The aim of this study is to determine how changes in bone mineral density (BMD) during pregnancy contribute to the changes in blood lead levels during pregnancy.

II. Lead

Sources of Lead Exposure

Common sources of lead among pregnant women include environmental, occupational, and dietary exposures such as living near smelters, using lead-based ceramics, being exposed to lead paint (3), and smoking cigarettes or being exposed to second-hand smoke (4, 5). Less common sources of lead exposure include consumption of nonfood items (pica) such as dirt, soap, and pottery. A New York Department of Health lead poisoning surveillance program from 1996 to 1999 reported several sources of lead among pregnant women with blood lead levels ≥20 μg/dL: 39% of women reported current pica intake (nonfood items such as soil, dirt, clay, pottery, or soap), 21% reported current use of imported pottery, 24% reported current consumption of imported goods (spices, tea, food), and 12% reported a history of exposure (e.g. from prior occupation, pica consumption, or use of ceramics) (6).

Those with higher lead exposures were more likely to be younger, foreign-born women; consume nonfood items; have limited access to culturally appropriate prenatal care; and have lower calcium intake (6). Additional factors associated with increased exposures to lead include smoking cigarettes, lower educational level, and lower socioeconomic status (2, 4, 5). Lead exposure in many developing countries is typically higher than in the U.S.

Changes in Lead Levels during Pregnancy

Lead has a half-life of approximately 45 days in the bloodstream, but a half-life of years when it resides in the bones, which is where 95% of the lead resides in the body (7, 8). In addition, increased mobilization of bone lead to maternal blood lead during pregnancy has been hypothesized in a previous cohort study examining the change in the

ratio of lead isotopes (9). Lead in plasma crosses the placenta via passive diffusion (8, 10, 11).

A cohort of pregnant women who received prenatal care at Magee-Women's Hospital in Pittsburgh, Pennsylvania between October 1992 and February 1995 was used in a prospective study that investigated the pattern of blood lead levels during pregnancy (12). Blood lead concentrations were collected once in each of the first and second trimesters and up to three times in the third trimester for a maximum of five measurements. Results from this study showed a U-shaped pattern of maternal blood lead levels during pregnancy where the lowest levels occur during second trimester when blood volume increases while the highest levels occur during third trimester when the calcium requirements of the developing fetus increase (12-14). This suggests that the fetus is continuously exposed to lead with the greatest exposure occurring during late pregnancy (15). The study further showed that pregnant women with low calcium intake (<600 mg/day) had higher levels of blood lead during the latter half of pregnancy compared to those with high calcium intake (>2,000 mg/day) (12). Thus, the study suggested a protective effect of calcium supplementation in lowering maternal blood lead levels. Additionally, higher maternal blood lead levels were associated with increased maternal age ($\beta = 0.37$, SE = 0.12) and with lower educational level ($\beta = -0.12$, SE = 0.07). Other predictors of increased maternal blood lead levels include higher caffeine intake, being African American, and smoking (12, 13, 16). Limitations of this study included self-reported measures of covariates such as daily calcium intake.

Another prospective study involving 105 pregnant women from the National Institute of Perinatology in Mexico City had five measurements of blood lead at 8-week

intervals from week 12 to week 36 of pregnancy and at delivery. The results from this study further supported the U-shaped pattern of maternal blood lead levels during pregnancy where there was a significant decrease in blood lead level between week 12 and week 20 ($p<0.05$) and a significant increase in blood lead level between week 20 and delivery ($p<0.01$) (13). Again, the increase in blood lead during the third trimester coincided with increased calcium requirements for the fetus.

A 2-year prospective study recruited 290 pregnant women living near a smelter in northern Sweden and 194 pregnant women living 120 km south of the smelter (reference area). These two residence locations were similar in terms of number of residents, social factors, and economic factors. Measurements of maternal blood lead concentrations were collected at week 10, week 32, and at delivery between February 1989 and February 1991. The mean blood lead levels of women living both near the smelter area and far from the area were the lowest during week 32 (Mean ± SD: 0.127 ± 0.051 μmol/L; 0.110 ± 0.040 μmol/L) and the highest at delivery (Mean ± SD: 0.159 ± 0.047 μmol/L; 0.138 ± 0.039 μmol/L). The blood lead levels significantly increased between week 32 of pregnancy and delivery regardless of decreases in environmental exposures to lead from smelter's emissions (17). Those living near a smelter had significantly higher blood lead levels ($p < .05$ at week 10, $p < .01$ at week 32, $p < .001$ at delivery) than those who did not (17). Although this study accounted for time and also found a U-shaped pattern of maternal blood lead levels during pregnancy, the study did not find a significant association between decreased calcium levels and increased blood lead levels nor were other factors associated with increased blood lead levels during pregnancy.

Several studies have suggested increasing calcium supplementation among women to lower maternal blood lead levels towards the later stages of pregnancy (12, 18, 19). In the Magee-Women's Hospital study population, higher calcium intake (>2,000 mg/day) was associated with lower blood lead levels between week 20 and delivery (-1.0% change in blood lead) (12). In a cross-sectional lead surveillance program of 1849 pregnant women recruited from various government hospitals in Mexico City between 1991-1993, maternal and umbilical cord blood samples were collected at delivery to investigate the effect of milk consumption on blood lead levels. This study reported an higher consumption of orange juice and milk was associated with decreased blood lead levels ($p \leq 0.01$). Blood lead levels of women who consumed more than 7 glasses of milk each week were 2.3 mg/dL lower, on average, than women who consumed less than 7 glasses of milk each week ($p < 0.01$) (18). Although this study supported a protective effect of calcium intake on maternal blood lead levels during pregnancy, its cross-sectional design only allowed one measurement of blood lead at delivery and thus, causality could not be clearly established.

In a prospective study that examined ten female immigrants to Australia, calcium supplementation were administered to the participants and monthly blood samples were taken during pregnancy to measure blood lead levels. This cohort of women with high calcium intake had lower mean blood lead level compared to a previous cohort of women with lower calcium intake examined by the same researchers(145 ug vs. 330 ug) (20). However, this study had a small sample size. Recent randomized placebo-controlled trials have further supported the protective effect of calcium supplementation, reporting 11%

average reductions in blood lead levels among women who took calcium supplementation daily compared to women taking placebo (21, 22).

A cross-sectional analyses at each trimester among a cohort of pregnant women in Mexico City reported increases in bone lead were associated with increases in plasma lead levels (patella lead: 9%, $p = 0.07$, 24%, $p < 0.01$, 25%, $p < 0.01$; tibia lead: 8%, $p = 0.16$, 19%, $p < 0.01$, 13%, $p = 0.01$ (23). In addition, lower calcium intake was associated with increased plasma lead levels (β =-0.02695% CI: -0.046, -0.005) (23). In contrast, a previous cross-sectional study from Magee-Women's Hospital reported no association between calcium intake and maternal blood lead or umbilical cord blood lead levels (24). However, maternal blood lead was observed at only one point (at delivery) and calcium intake was based on self-reported milk intake only. In summary, recent studies have hypothesized that maternal blood lead levels increase during later stages of pregnancy and suggested that there is a protective effect of calcium intake among pregnant women in reducing maternal blood lead levels, which would lower fetal exposure to lead.

Transfer of Lead from Mother to Fetus

Measurement of Lead (Plasma vs. Whole Blood)

Past studies involving rats have reported high correlation between fetal and maternal blood lead levels (r = 0.77-0.80), suggesting a lack of placental-fetal barrier to lead (10). Current research suggests that lead crosses the placenta via passive diffusion in humans as well beginning around 12-14 weeks of gestation (10, 25). However, there continues to be a lack of data on intrauterine lead toxicity to the nervous system among both animal and human fetuses (10). Lead that crosses the placenta via passive diffusion is from lead within blood plasma. However, fetal lead exposure is typically measured

from umbilical cord blood (whole blood lead levels) that mostly captures lead found in red blood cells (>99%) rather than lead found in blood plasma (<1%) (11). A longitudinal study among 237 environmentally exposed pregnant women living in Mexico City that investigated the relation between blood lead and plasma lead levels found that plasma lead varied within women independently of changes in whole blood lead (15). Although measuring lead levels from whole blood (i.e. umbilical cord blood) may be an inaccurate estimate of fetal exposure, using plasma lead levels as a biomarker to determine fetal lead exposure during pregnancy has not yet been done due to the high costs of instruments used in measuring low lead levels in blood plasma (< 0.1 μg/dL). In addition, red blood cells may break down and contaminate plasma lead (11, 15).

In the study of pregnant women in Mexico City, a curvilinear relationship was found between blood plasma and whole blood lead levels where blood plasma lead levels increased with gestational age relative to whole blood lead levels (15). This finding further suggested that, on average, blood plasma lead levels would continue to increase during second and third trimesters even if lead levels in whole blood (>140 μg/L) were to remain unchanged (15). Thus, it has been hypothesized that fetal lead exposure during later stages of pregnancy is much higher than indicated based on whole blood lead.

In the Magee-Women's Hospital study, higher cord blood lead levels relative to maternal blood lead were reported among women older than 30 years of age with higher blood pressure compared to women younger than 30 years of age with lower blood pressure (β=2.66, 95% CI: -0.12, 5.43) and among women who reported alcohol consumption during third trimester compared to those who did not (β=1.92, 95% CI: 0.76, 3.09) (24). Additionally, biological factors such as having higher maternal

hemoglobin and sickle cell trait were associated with lower cord blood lead levels compared to maternal blood lead (β=-0.12, 95% CI: -0.22, -0.02; β=-0.74, 95% CI: -1.17, -0.30), respectively (24). A limitation of this study was that blood lead was analyzed at only one point in time, which prevents the study from drawing any causal relationships.

Studies on adverse effects of prenatal lead exposure

In-utero exposure to lead in human fetuses has been well-documented in recent studies that have shown associations between maternal lead levels during pregnancy and various adverse outcomes in children (24, 26-29). In a nested case-control study among a cohort of pregnant women in Mexico City (1994-1996), maternal blood lead levels were measured during the first 12 weeks of gestation. The risk of spontaneous abortion (before 21 weeks of gestation) increased linearly as the amount of lead exposure increased from low to moderate (5-9, 10-14, and ≥15 μg/dL) compared to the reference group (<5 μg/dL), (OR = 2.3, 5.4 12.2; test for trend, p = 0.03) (26).

Baseline data from a randomized double-blind calcium supplementation trial study among lactating women was used to investigate the relationship between lead burden and birth weight. Samples of cord blood lead levels at delivery and maternal bone lead levels (tibia and patella) at 1-month postpartum were collected among 272 mother-infant pairs in Mexico City. This study found that higher postpartum maternal tibia lead levels were associated with lower birth weight (p = 0.003) (27, 28). Umbilical cord blood lead was also found to be inversely associated with birth weight, but the association was not statistically significant (p = 0.54) (27). The main limitation of this study was that bone lead was measured after pregnancy.

In a prospective study among pregnant women from prenatal clinics at the National Institute of Perinatology in Mexico City, maternal blood lead levels were measured every 8 weeks between gestational weeks 12 and 36 and once at delivery. Higher maternal blood lead at week 36 of pregnancy was associated with smaller head circumferences (cm) among 6-month old infants (β=0.27, 95% CI: 0.02, 0.32) (30). In another prospective study using this same cohort, higher maternal blood lead levels during the third trimester (approximately week 28) was associated with lower child IQ (β=-3.90, 95% CI: -6.45, -1.36) (29).

III. BMD

BMD Changes during Pregnancy

Rates of maternal bone turnover are high during pregnancy with BMD decreasing as bone resorption increases during late pregnancy (31). The fetus requires approximately 30 g of calcium from maternal dietary intake, absorption, and bone stores during pregnancy and thus, stores of calcium in addition to lead in the bone are released into the bloodstream during bone resorption (13, 18, 20, 32). Consequently, past studies have suggested adequate calcium intake via diet and supplements are an important factor in reducing maternal bone resorption as well as BMD loss during late stages of pregnancy (33-35). Thus, blood lead levels would vary depending on the amount of bone lead released into plasma.

Maternal BMD is typically measured at the lumbar spine, hip (trochanter), and distal forearm with dual-energy x-ray absorptiometry (DEXA) throughout pregnancy and its values are reported in g/cm^2 and percentage change from baseline (31, 36). In the Magee-Women's Hospital study, BMD of radius and ulna at three forearm sites (1/3-

distal, mostly cortical bone; mid-distal, a mix of trabecular and cortical bone; and ultra-distal, primarily (95%) trabecular bone) were measured at approximately weeks 16 and 36 of pregnancy. The mean BMD loss at ultra-distal was 1.9% per 20 weeks (95% CI: 1.2-2.5) and the odds of losing ≥ 5% of ultra-distal BMD were 2.3 times higher (95% CI: 0.7-7.7) between 16 and 36 weeks of gestation for those who had lower calcium intake (< 1000 mg/day) compared to those with higher calcium intake (≥ 2000 mg/day) (34). Long-term consequences of BMD loss during pregnancy include fractures and accelerated postmenopausal BMD loss, which may lead to osteoporosis (37).

A prospective study that observed rates of bone resorption using biochemical markers of urine among ten women during pregnancy reported increased rates of bone resorption starting in week 14 ($p < 0.02$) and peaking at week 38 ($p < 0.001$) (36). This study also measured BMD of hip and spine before pregnancy and at 6 weeks postpartum as well as forearm BMD before pregnancy, at 14 weeks, at 28 weeks, and at 6 weeks postpartum. Results suggested that BMD of hip and spine decreased significantly ($p < 0.05$) while the decrease in forearm BMD was of similar magnitude but not statistically significant (Table 1) (36). Several studies have reported varying results of BMD loss due to measurements taken at different sites of bone and during different gestational ages. Changes in BMD were typically measured between preconception (baseline) and postpartum. Table 1 includes results from studies that have found BMD loss (34, 36-43), increase (43), or no change (44-48) during pregnancy or postpartum. BMD loss seems to occur mostly during late pregnancy regardless of bone sites. In contrast, increases in BMD are reported most frequently when measurements are taken between pre-pregnancy

and postpartum. Overall, the research findings have been consistent in reporting BMD loss during pregnancy.

Risk Factors for Decreased Bone Mineral Density (BMD)

Currently, there are a limited number of studies that have investigated the risk factors for bone loss during pregnancy. The main risk factors that have been examined include early pregnancy weight (β = 0.383, p = 0.409), height (β = -0.202, p = 0.388), early pregnancy body mass index (β = -0.360, p = 0.418), total weight gain (β = 0.021, p = 0.561), total fat accumulation (β = -0.08, p = 0.033), and initial BMD value (β = 0.383, p = 0.001) (49). Logistic regression analyses used for BMD loss found additional associations with maternal age (ages 18-20: OR=5.4, 95% CI: 1.6-17.9; ages 21-30: OR=1; ages 31-41: OR=6.4, 95% CI: 1.9-21.6), lower calcium intake (< 1000 mg/day vs. ≥ 2000 mg/day) (OR=2.3, 95% CI: 0.7-7.7) (34, 35), regular exercise (any vs. none) (OR=0.7, 95% CI: 0.2-1.8), and bed rest prescribed during pregnancy (yes vs. no) (OR=6.5, 95% CI: 2.2-18.9) (34).

IV. Conclusion

Previous research has focused on changes in BMD and changes in blood lead levels during pregnancy separately. Additionally, there are a limited number of studies that have looked at either the changes in BMD or blood lead levels longitudinally over various time points across pregnancy. Although a previous cross-sectional study investigated the relation between BMD and lead levels in blood, tibia, and patella and found a positive association between bone (tibia) lead levels and BMD (p = 0.04), this study was done among women 50-70 years of age (50). There has yet to be a longitudinal study exploring the relationship between change in BMD and change in blood lead levels

during pregnancy. A longitudinal study in Mexico City has reported that pregnant women with both higher bone lead levels and higher bone resorption had higher plasma lead levels (23); however, changes in bone mineral density across pregnancy were not measured in this study.

The primary objective of this study is to examine the relation between changes in BMD and changes in maternal blood lead levels across pregnancy among women who received prenatal care during their first 12 weeks of pregnancy at Magee-Women's Hospital in Pittsburgh, Pennsylvania from 1992 to 1995. This cohort of women had their blood lead levels measured up to five times across their pregnancies as well as having their BMD measured at gestational weeks 16 and 36, on average. An important public health implication from previous research findings is that calcium supplementation is associated with both decreased blood lead levels and smaller decreases in BMD during pregnancy. This study will evaluate whether self-reported calcium intake confounds the association between changes in BMD and blood lead levels. If so, this may support the value of calcium supplementation for pregnant women. Reducing prenatal lead exposure is important for reducing adverse outcomes associated with lead.

Table 1. BMD Changes in Various Studies

Authors	Study Design	Sample Size	Primary Objective	BMD Measurement	BMD % Change	Limitations
Bjorklund et al. (1999)	Prospective	n = 49	association between pregnancy-related back and pelvic pain and changes in bone density	distal and ultra-distal forearm at weeks 12 and 35 of pregnancy and at 5 months post partum	1.1% decrease at cortical bone ($p<0.001$) between week 35 of pregnancy and at 5 mos postpartum; 0.6% decrease at trabecular bone	not measured during pregnancy
Black et al. (2000)	Prospective	n = 10	effect of normal pregnancy on the human skeleton; bone turnover before, during, and after pregnancy	hip and spine before pregnancy and at 6 weeks postpartum; forearm BMD (1/3-distal, mid, ultra-distal) before pregnancy, at 14 weeks, at 28 weeks, and at 6 weeks postpartum	3.5% decrease at spine ($p<0.05$), 2.0% decrease at total hip ($p<0.05$), 4.8% decrease at femoral neck ($p<0.01$), 3.3% decrease at trochanter ($p<0.05$), 3.4% decrease at Ward's triangle, 2.3% decrease at mid-distal, 4.2% decrease at 1/3-distal, 3.1% decrease at ultra-distal	no significant changes in forearm BMD when measured throughout pregnancy
Cross et al. (1995)	Prospective	n = 10	effects of stages of reproduction on BMD and markers for bone turnover	1/3-distal and ultra-distal radius of dominant arm at pre-pregnancy, first, second, third trimester, 3 mos lactation, and postweaning	0.9% decrease at ultra-distal, 0.3% decrease at 1/3-distal (across trimesters only)	
Gambacciani et al. (1995)	Prospective	n = 85	pattern of bone density during pregnancy	os calcis at every 4 weeks between 14 and 38 weeks of pregnancy using ultrasonography	1.2% decrease between weeks 14 and 38 ($p<0.05$)	

Honda et al. (1998)	Longitudinal	n = 111	relationship between change of bone metabolism in lumbar trabecular and serum hormonal changes in pregnancy and lactation	lumbar spine at 3-5 days post-delivery, 3 and 6 months postpartum	5% decrease among lactating women at 3 months compared to BMD at 3-5 days postpartum, 6% decrease among lactating women at 6 months postpartum compared to BMD at 3-5 days postpartum ($p<0.001$)	not measured during pregnancy
Kaur et al. (2003)	Prospective	n = 46	longitudinal changes in BMD during pregnancy	lumbar spine and hip (femoral neck, trochanter, total hip) before conception and within 2 weeks of completion to full-term pregnancy	0.9% decrease at lumbar spine, 1.2% decrease at total hip, 0.7% decrease at femoral neck, 4.2% decrease at trochanter	
Kent et al. (1993)	Prospective	n = 37	to measure serially the maternal intake and efficiency of Ca absorption, urinary Ca excretion and FBMD	3 forearm radio-ulnar sites of distal forearm at week 14 & week 36 during pregnancy, at weeks 6 & 24 during lactation, and at 8/24 weeks weaning	no change during pregnancy	BMD change only seen during lactation
Kolthoff et al. (1998)	Prospective	n = 59	influence of pregnancy, lactat ion and weaning on bone mineral de nsity	non-dominant radius ultra distally and more proximally in weeks 18 and 37 of pregnancy, and 0, 3, 6, 12 and 18 months after delivery; lumbar spine & proximal femur all after delivery	2% decrease at ultra-distal during pregnancy	
Matsumoto et al. (1995)	Prospective	n = 22	effects of pregnancy and postpartal lactation on bone mineral density (BMD)	distal radius of the forearm at 8 points in time between first trimester of pregnancy to 24 months postpartum	no change during pregnancy	BMD change only seen postpartum during lactation

More et al. (2001)	Prospective	n = 38	monitor the changes in BMD during pregnancy, lactation and postweaning in a prospective manner	lumbar spine and forearm [distal 33% & ultradistal region of the radius] within 3 months before conception (baseline), between weeks 22 and 24 of pregnancy, within 6 days of delivery, and at 6 & 12 months postpartum	3.9% decrease at distal (%±SD at gestation: 100.1±0.8, %±SD at delivery: 96.1.1±2.1) and 3.2% decrease at ultra-distal (%±SD at gestation: 99.4.1±1.35, %±SD at delivery: 96.2.1±1.6) between 22-24 weeks gestation and delivery; 2.1% decrease at lumbar spine (%±SD at baseline: 100, %±SD at delivery: 97.9±1.6), 3.8% decrease at distal (%±SD at baseline: 100) and 3.8% decrease at ultra-distal %±SD at baseline: 100) between baseline and delivery	only one point during pregnancy (22-24 weeks)
Nayor et al. (2000)	Prospective	n = 16	evaluate the changes in bone density and bone turnover during pregnancy	before pregnancy; then at 16, 26, and 36 weeks of pregnancy; and postpartum	4.5% decrease at spine (SE 1.5%), 3.2% decrease at pelvis (SE 1.4%); increase at cortical bone sites: 2.8% in arms (SE 1.0%), 1.8% in legs (SE 0.5%) (between baseline and postpartum)	
Promislow et al. (2004)	Prospective	n = 181	evaluate trabecular and cortical bone changes during pregnancy	3 forearm sites: 1/3-distal, mostly cortical bone; mid-distal, a mix of trabecular & cortical bone; and ultra-distal, primarily (95%) trabecular bone at average gestational ages of 16 and 36 weeks	1.9% decrease at ultra-distal (95% CI 1.2-2.5)	

Ritchie et al. (1998)	Prospective	n = 14	effect of pregnancy on spinal trabecular and total-body bone mineral	total body, arms, legs, trunk (sternum, ribs, spine, pelvis), lumbar spine (trabecular BMD) at pre-pregnancy and 1-2 week post-delivery, 2mos postpartum, post-menses	arms: 0.886 ± 0.055 g/cm^2 pre-pregnancy, 0.890 ± 0.051 g/cm^2 post-delivery; legs: 1.204 ± 0.071 g/cm^2 pre-pregnancy, 1.211 ± 0.075 g/cm^2 post-delivery; trunk: 0.941 ± 0.064 g/cm^2 pre-pregnancy, 0.935 ± 0.063 g/cm^2 post-delivery; total body: 1.156 ± 0.055 g/cm^2 pre-pregnancy, 1.162 ± 0.055 g/cm^2 post-delivery; mostly increases	not measured during pregnancy
Sowers et al. (1991)	Prospective	n = 32	determine association between pregnancy and femoral bone loss	femoral neck, trochanter, wards triangle before conception and within 15 days of parturition	femoral neck: 0.011 ± 0.05 g/cm^2, trochanter: -0.009 ± 0.04 g/cm^2, Ward's triangle: 0.021 ± 0.07 g/cm^2	not measured during pregnancy

CHAPTER II: DECREASE IN BONE MINERAL DENSITY DURING PREGNANCY AND CHANGE IN MATERNAL BLOOD LEAD LEVELS

Heejoo Jo, Penelope P. Howards, Irva Hertz-Picciotto

ABSTRACT: Prenatal exposure to lead has been associated with spontaneous abortion, low birth weight, and developmental delay. Lead stored in women's bones is released into their blood during pregnancy. Maternal blood lead can cross the placenta and expose the fetus. The purpose of this study is to examine the relation between change in bone mineral density (BMD) and change in maternal blood lead levels across pregnancy. Additionally, this study evaluated whether the association between changes in BMD and blood lead is confounded by self-reported daily calcium intake. This was a prospective study of 100 women who received prenatal care during the first 12 weeks of pregnancy at Magee-Women's Hospital in Pittsburgh, Pennsylvania from October 1992 to February 1995. Change in maternal blood lead was calculated using the difference between blood lead levels (μg/dL) measured, on average, at 17 and 37 weeks gestation. Change in BMD was calculated using the difference between measurements of the ultra-distal BMD at 16 and 36 weeks gestation. Information regarding sociodemographic and lifestyle factors was collected during interviews between 8 and 18 weeks gestation and again between 31 and 41 weeks gestation. As expected, maternal blood lead levels increased and ultra-distal BMD decreased during pregnancy. However, change in BMD was weakly associated with change in maternal blood lead levels during pregnancy ($\beta = 0.33$ μg/dL, 95% CI = -8.45, 7.80) in a linear regression model adjusted for age, race, smoking, BMI, and calcium intake. Change in blood lead was higher among women whose calcium intake was <1,000 mg/day compared to 1,000-<2,000 mg/day. These findings suggest that the observed increase in maternal blood lead during pregnancy may not be predominantly due to the increased mobilization of bone lead as previously hypothesized. Further research on the mechanisms that contribute to increased maternal blood lead during pregnancy is needed.

Introduction

The effects of exposure to lead have been studied extensively due to the persistence of lead in the environment. Recent studies suggest that prenatal exposure to lead leads to spontaneous abortion (26), low birth weight (27, 28), and smaller head circumferences among infants (30). Additionally, children with increased blood lead levels have displayed developmental delays and lower intelligence (1). Lead stored in bone may be released into maternal blood during pregnancy, and lead in maternal blood can cross the placenta via passive diffusion and expose the fetus (8, 10, 11).

Lead has a half-life of approximately 45 days in the bloodstream, but a half-life of years when it resides in the bones, which is where 95% of the lead resides in the body (7, 8). Bone lead levels reflect cumulative lead exposure over a period of time, but may be released into plasma during bone turnover. Rates of maternal bone turnover are high during pregnancy with bone mineral density (BMD) decreasing as bone resorption increases during late pregnancy (31). The fetus requires approximately 30 g of calcium from maternal dietary intake, absorption, and bone stores during pregnancy and thus, calcium in addition to lead in the bone is released into the bloodstream during bone resorption (32). Consequently, past studies have suggested adequate calcium intake via diet and supplements is an important factor in reducing maternal bone resorption as well as BMD loss during late stages of pregnancy (33-35).

Several studies have reported a U-shaped pattern for maternal blood lead levels during pregnancy with lead levels decreasing around the second trimester when blood volume increases, and increasing around the third trimester when calcium requirements

of the fetus increase (12-14, 17). This suggests that the fetus is continuously exposed to lead with the greatest exposure occurring during late pregnancy (15).

Several cross-sectional studies report calcium supplementation is associated with lower maternal blood lead levels late in pregnancy (12, 18, 19, 23). In contrast, a cross-sectional study from Magee-Women's Hospital reported no association between calcium intake and maternal blood lead or umbilical cord blood lead levels (24). The primary limitation in these studies is that maternal blood lead was observed at only one point in time. However, in a prospective study at Magee-Women's Hospital, higher calcium intake (>2,000 mg/day) was associated with lower blood lead levels between week 20 and delivery (12). This finding was further confirmed in another prospective study among female immigrants in Australia where women with higher calcium intake had lower mean blood lead level compared to a previous cohort of women with lower calcium intake examined by the same researchers (145 ug vs. 330 ug) (20). Further, recent randomized placebo-controlled trials have supported the protective effect of calcium supplementation, reporting overall 11% average reductions in blood lead levels among women who took calcium supplementation daily compared to women taking placebo (21, 22). Overall, recent studies have hypothesized that maternal blood lead levels increase during later stages of pregnancy and that there is a protective effect of calcium intake among pregnant women in reducing maternal blood lead levels, and thus lowering fetal exposure to lead.

The primary objective of this study is to examine the relation between change in BMD and change in maternal blood lead levels during pregnancy among women who received prenatal care during their first 12 weeks of pregnancy at Magee-Women's Hospital in Pittsburgh, Pennsylvania from 1992 to 1995. This study will also evaluate

whether the association between changes in BMD and blood lead levels is confounded by self-reported daily calcium intake.

Methods

Study Population

A cohort of pregnant women were recruited from Magee-Women's Hospital in Pittsburgh, Pennsylvania from 1992 to 1995 (12). Participants were eligible if they started receiving prenatal care during their first 12 weeks of pregnancy, were 18 years of age or older, were English-speakers, were planning to carry the pregnancy to term, were African American or White, and were free of any preexisting chronic medical conditions.

A total of 753 women were initially enrolled. For this study, only the subset of women who had blood lead concentrations determined once in each of the second and third trimesters was considered. This exclusion decreased the sample size to 178 women. Among these women, only those who had BMD measurements using the same arm during both the second and third trimesters were included in this study. The final study sample consisted of 100 women.

Change in maternal blood lead levels

Maternal blood lead levels (μg/dL) were measured once in both the first and second trimesters of pregnancy, up to three times during the third trimester, and at delivery (12). Blood lead analyses were performed by the San Francisco General Hospital Metals Laboratory, University of California, San Francisco, using graphite furnace atomic absorption spectrophotomery with deuterium background correction. For this study, the change in blood lead variable was calculated by taking the difference between blood lead levels measured, on average, at 37 and 17 weeks gestation. These gestational

weeks were chosen because they coincided most closely with the gestational weeks that BMD was measured during pregnancy.

Change in bone mineral density

Bone mineral density (g/cm^2) of the radius and ulna was measured at three forearm locations: the 1/3-distal (mostly cortical bone); the mid-distal (a mix of trabecular and cortical bone); and the ultra-distal (primarily (95%) trabecular bone) at 16 and 36 weeks gestation (34). A DXA instrument (Hologic QDR-1000, Waltham, Mass) that was calibrated daily using a phantom probe was used to measure bone mineral density (BMD) (34). For this study, the change in BMD variable was calculated using the difference between second and first measurements of the ultra-distal BMD measurement. In this study population, the greatest BMD loss occurred at the ultra-distal site (34).

Covariates

Information regarding sociodemographic and lifestyle factors was collected during interviews between 8 and 18 weeks gestation and again between 31 and 41 weeks of pregnancy (12). These factors included age, race, education level, current smoking status, alcohol intake before pregnancy, exercise, and diet. Self-reported height and pre-pregnancy weight were used to calculate pre-pregnancy body mass index (BMI) of each woman. Additionally, calcium intake (mg/day) was obtained from self-reported information on milk consumption, dietary calcium intake from various food items, and supplemental calcium intake from prenatal vitamins, calcium supplements, and antacids. For this study, total calcium intake was calculated by combining all the sources of calcium at each interview. Calcium intake reported at each interview was then averaged

and categorized into three levels: <1000 mg/day (below recommended intake), 1000-<2000 mg/day, and ≥2000 mg/day (above recommended intake).

Statistical analyses

Descriptive statistics for both maternal blood lead values (μg/dl) and BMD (g/cm^2) were performed overall and stratified by time of measurement during pregnancy for each sociodemographic or lifestyle characteristic. For these analyses, age at enrollment was categorized based on tertiles, current smoking status was dichotomized into ever smoked vs. never smoked, and body mass index (kg/m^2) before pregnancy was categorized based on World Health Organization (WHO) principal cut-off points. In addition, change in blood lead was plotted against change in BMD.

Multiple variable linear regression analysis was conducted using change in blood lead levels as the dependent variable and change in BMD as the main exposure. Potential confounders were assessed based on the literature, a directed acyclic graph (DAG), and bivariate linear regressions. Age at enrollment was the only factor that was significantly associated with both exposure and outcome. However, calcium intake was of particular interest as a potential confounder in this study and thus, included in the final model. Age, race, education, smoking status, BMI were also included in the final model based on the DAG and the prior literature. Violations of assumptions were checked through partial plots of each independent variable, a residual plot, a normal probability plot, and a histogram of the residuals. There were no gross violations of the linearity, homoscedasticity, or normality assumptions. All analyses were done using SAS version 9.3, SAS Institute, Cary, NC.

Results

Median maternal blood lead values (μg/dL) are presented by sociodemographic and lifestyle characteristics in Table 1. Median blood lead was higher towards the end of third trimester (median = 1.80 μg/dL, IQR = 0.85) compared to second trimester (median = 1.45 μg/dL, IQR = 0.85) overall and for all covariates. During the third trimester, blood lead levels increased with age. Blood lead was also higher among African American women, women who had ever smoked, and among women who were overweight or obese. Women who consumed calcium below the recommended daily intake had lower blood lead than women consuming the recommended amount or more during the second trimester. In contrast, women who consumed more than the recommended daily intake had lower blood lead than those consuming the recommended amount or less during the third trimester.

Blood lead levels increased during pregnancy for all factors with an overall increase of 0.30 μg/dL. Women who were ages 18-20 had a smaller change in blood lead (median = 0.20 μg/dL) than older women. Change in blood lead increased as BMI increased although the changes were small. The change in blood lead was smaller in women who currently exercised compared to those who did not. Change in blood lead decreased as calcium intake increased.

The ultra-distal BMD was lower at 36 weeks gestation (median = 0.45 g/cm^2, IQR = 0.05) compared to 16 weeks gestation (median = 0.46 g/cm^2) overall and across all covariates (Table 2). Although BMD did not vary much across covariates, BMD at each trimester was higher among African Americans, women with less than high school education, women who had ever smoked, and women who were not currently exercising.

The decrease in BMD was smaller with increasing age, and women who ever smoked had a larger decrease in BMD than women who never smoked. As BMI increased, the decrease in BMD was larger (normal: median = -0.006 g/cm^2; overweight: median = -0.009 g/cm^2; obese: median = -0.016 g/cm^2). BMD decreased more among those who had <1,000 mg/day calcium intake (median = -0.015 g/cm^2, IQR = 0.014) compared to those who had either 1,000-<2,000 mg/day calcium intake or ≥2,000 mg/day.

No association was apparent when change in blood lead was plotted against change in BMD (Figure). Results from an unadjusted linear regression model indicated a one unit increase in change in BMD was associated with a 0.24 μg/dL increase in change in blood lead (95% CI = -7.33, 7.80). However, after adjusting for age, race, smoking, BMI, and calcium intake, a one unit increase in change in BMD was associated with a 0.33 μg/dL decrease in change in blood lead (95% CI = -8.45, 7.80), on average. This association become stronger (β = -0.73, 95% CI = -8.87, 7.41) when calcium intake was removed from the model. The average change in blood lead was increased by 0.28 μg/dL (95% CI = -0.08, 0.64) among women whose calcium intake is <1,000 mg/day compared to 1,000-<2,000 mg/day while there was no association between change in blood lead and calcium intake ≥2,000 mg/day versus 1,000-<2,000 mg/day (β = -0.01, 95% CI = -0.30, 0.27).

Discussion

Findings from the adjusted analysis support the hypothesis that decreasing BMD across pregnancy is associated with increasing maternal blood lead. However, the association is weak and imprecise. Additionally, calcium intake below the recommended daily intake is associated with increased blood lead compared with the recommended

calcium intake (12), and calcium intake does appear to confound the association between change in BMD and change in blood lead.

Previous studies have reported increasing maternal blood lead levels during late pregnancy (12, 13, 15, 17), which is consistent with our findings. Other studies have reported that BMD decreases during pregnancy (37, 39, 41-43), which is also consistent with our data. Reported BMD loss varies due to measurements taken at different sites of bond and during different gestational ages. However, BMD losses are observed mostly during late pregnancy regardless of bone sites. Gulson et al. estimated the contribution of mobilized bone lead to maternal blood lead during pregnancy by examining the change in the ratio of lead isotopes common in a previous environment to lead isotopes common in the current environment of recent immigrants. While the ratio decreased over time prior to pregnancy, the ratio increased after pregnancy, which the authors interpreted as an indication of increased mobilization of bone lead (9). This study is the first to simultaneously examine the relation between change in BMD and change in maternal blood lead across pregnancy. After adjusting for confounders, we observed an association in the expected direction, but it was small and our results were imprecise.

The protective effect of calcium supplementation observed in this analysis is consistent with previous findings of an association between increased calcium intake and decreased blood lead levels (12). Calcium intake was based on self-report, but it did incorporate multiple sources of calcium and the reported calcium intake was consistent across interviews (data not shown).

The primary limitation of this study is the small sample size. Only 100 women had both ultra-distal BMD measurements across pregnancy using the same arm and had

measured blood lead levels during gestational weeks 17 and 37. Confounding may also be an issue in this study. Common factors associated with blood lead among populations without occupational exposure to lead such as age, race, and smoking were included in the analysis, but there may be other unmeasured factors that confound the relationship between change in blood lead and change in BMD during pregnancy.

The observed increase in maternal blood lead between gestational weeks 17 and 37 may not be predominantly due to the increased mobilization of bone lead to blood lead as previously suggested (9). However, results from this study (though imprecise) confirmed an increase in maternal blood lead and decrease in ultra-distal BMD during late pregnancy. This study further suggests that calcium intake may provide some protection against increased maternal blood lead levels during pregnancy.

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TABLES

Table 1. Maternal blood lead values (µg/dL) based on sociodemographic and lifestyle characteristics among subset of pregnant women who had both measurements from second and third trimesters, Pittsburgh, Pennsylvania, 1992-1995[1]

		Second Trimester Blood Lead[a]			Third Trimester Blood Lead[b]			Change in Blood Lead[c]		
	n	median	Q1 (25%)	Q3 (75%)	median	Q1 (25%)	Q3 (75%)	median	Q1 (25%)	Q3 (75%)
Overall	100	1.45	1.10	1.95	1.80	1.40	2.25	0.30	-0.013	0.50
Age (years)										
18-20	33	1.35	1.10	1.80	1.55	1.00	2.10	0.20	-0.15	0.45
21-26	27	1.60	1.10	2.15	1.68	1.35	2.50	0.35	0.05	0.50
27-41	32	1.43	1.18	1.95	1.93	1.53	2.23	0.35	0.03	0.68
Race										
African American	53	1.60	1.20	2.10	1.90	1.50	2.45	0.35	0.05	0.50
White	46	1.40	1.05	1.85	1.68	1.35	2.20	0.30	-0.05	0.50
Education										
<High school	54	1.48	1.10	2.00	1.90	1.40	2.40	0.33	-0.05	0.60
≥High school	46	1.45	1.10	1.80	1.70	1.35	2.05	0.30	0.00	0.45
Smoking										
Ever	29	1.60	1.25	2.15	2.20	1.55	2.40	0.35	-0.05	0.60
Never	71	1.40	1.10	1.85	1.70	1.30	2.20	0.30	0.00	0.45
Drank alcohol before pregnancy										
Yes	70	1.48	1.15	1.95	1.80	1.40	2.25	0.30	-0.025	0.50
No	30	1.40	1.10	1.95	1.75	1.40	2.50	0.34	0.05	0.50
Body mass index (kg/m^2)										
18.5-<25 (normal)	50	1.35	1.10	1.80	1.68	1.40	2.20	0.30	0.00	0.50
25-<30 (overweight)	23	1.60	1.20	2.15	2.00	1.55	2.35	0.33	-0.05	0.45
≥30 (obese)	26	1.60	1.10	2.25	1.95	1.35	2.60	0.35	0.05	0.58
Current Exerciser										
Yes	54	1.50	1.10	1.95	1.80	1.45	2.20	0.25	-0.05	0.50
No	45	1.45	1.15	1.95	1.80	1.35	2.25	0.35	0.05	0.50
Calcium intake (mg/day)										
<1,000	16	1.23	1.08	1.78	1.88	1.45	2.45	0.43	0.23	0.69
1,000-<2,000	44	1.50	1.24	1.98	1.90	1.44	2.25	0.35	-0.06	0.56
≥2,000	36	1.45	1.05	1.93	1.68	1.33	2.21	0.23	-0.05	0.35

[1]Those who have blood lead values from visits 2 and 5, have both BMD measurements, same arm used for BMD measurements

[a]Measured at 17 weeks gestation, on average

[b]Measured at 37 weeks gestation, on average

[c]Difference between third and second trimester blood lead visits

Table 2. Ultra-distal BMD (g/cm^2) based on sociodemographic and lifestyle characteristics among subset of pregnant women who had both blood lead measurements from second and third trimesters, Pittsburgh, Pennsylvania, 1992-1995[1]

		First ultra-distal BMD[a]			Second ultra-distal BMD[b]			Change in BMD[c]		
	n	median	Q1 (25%)	Q3 (75%)	median	Q1 (25%)	Q3 (75%)	median	Q1 (25%)	Q3 (75%)
Overall	100	0.46	0.43	0.48	0.45	0.42	0.47	-0.009	-0.020	0.000
Age (years)										
18-20	33	0.47	0.43	0.49	0.44	0.43	0.47	-0.015	-0.027	-0.006
21-26	27	0.46	0.44	0.49	0.45	0.43	0.47	-0.011	-0.017	-0.001
27-41	32	0.45	0.42	0.47	0.45	0.42	0.48	-0.005	-0.017	0.004
Race										
African American	53	0.47	0.43	0.50	0.46	0.42	0.49	-0.010	-0.018	-0.001
White	46	0.45	0.43	0.47	0.44	0.42	0.46	-0.006	-0.021	0.000
Education										
<High school	54	0.47	0.44	0.49	0.45	0.43	0.49	-0.008	-0.018	-0.001
≥High school	46	0.45	0.43	0.47	0.44	0.41	0.46	-0.011	-0.023	0.001
Smoking										
Ever	29	0.47	0.44	0.49	0.45	0.44	0.49	-0.004	-0.015	0.002
Never	71	0.45	0.43	0.48	0.44	0.42	0.47	-0.011	-0.023	-0.001
Drank alcohol before pregnancy										
Yes	70	0.46	0.43	0.47	0.44	0.42	0.48	-0.009	-0.017	-0.001
No	30	0.46	0.44	0.49	0.45	0.43	0.47	-0.016	-0.026	0.001
Body mass index (kg/m^2)										
18.5-<25 (normal)	50	0.45	0.42	0.47	0.44	0.42	0.46	-0.006	-0.017	0.001
25-<30 (overweight)	23	0.46	0.44	0.49	0.46	0.42	0.49	-0.009	-0.017	-0.001
≥30 (obese)	26	0.47	0.45	0.52	0.45	0.43	0.51	-0.016	-0.024	-0.006
Current Exerciser										
Yes	54	0.45	0.42	0.47	0.44	0.41	0.47	-0.010	-0.020	0.000
No	45	0.47	0.44	0.49	0.45	0.43	0.48	-0.009	-0.017	-0.001
Calcium intake (mg/day)										
<1,000	16	0.44	0.41	0.47	0.44	0.40	0.45	-0.015	-0.021	-0.007
1,000-<2,000	44	0.46	0.44	0.49	0.46	0.42	0.48	-0.007	-0.017	0.001
≥2,000	36	0.46	0.43	0.48	0.44	0.42	0.46	-0.007	-0.022	0.001

[1]Those who have blood lead values from visits 2 and 5, have both BMD measurements, same arm used for BMD measurements

[a]Measured at 16 weeks gestation, on average

[b]Measured at 36 weeks gestation, on average

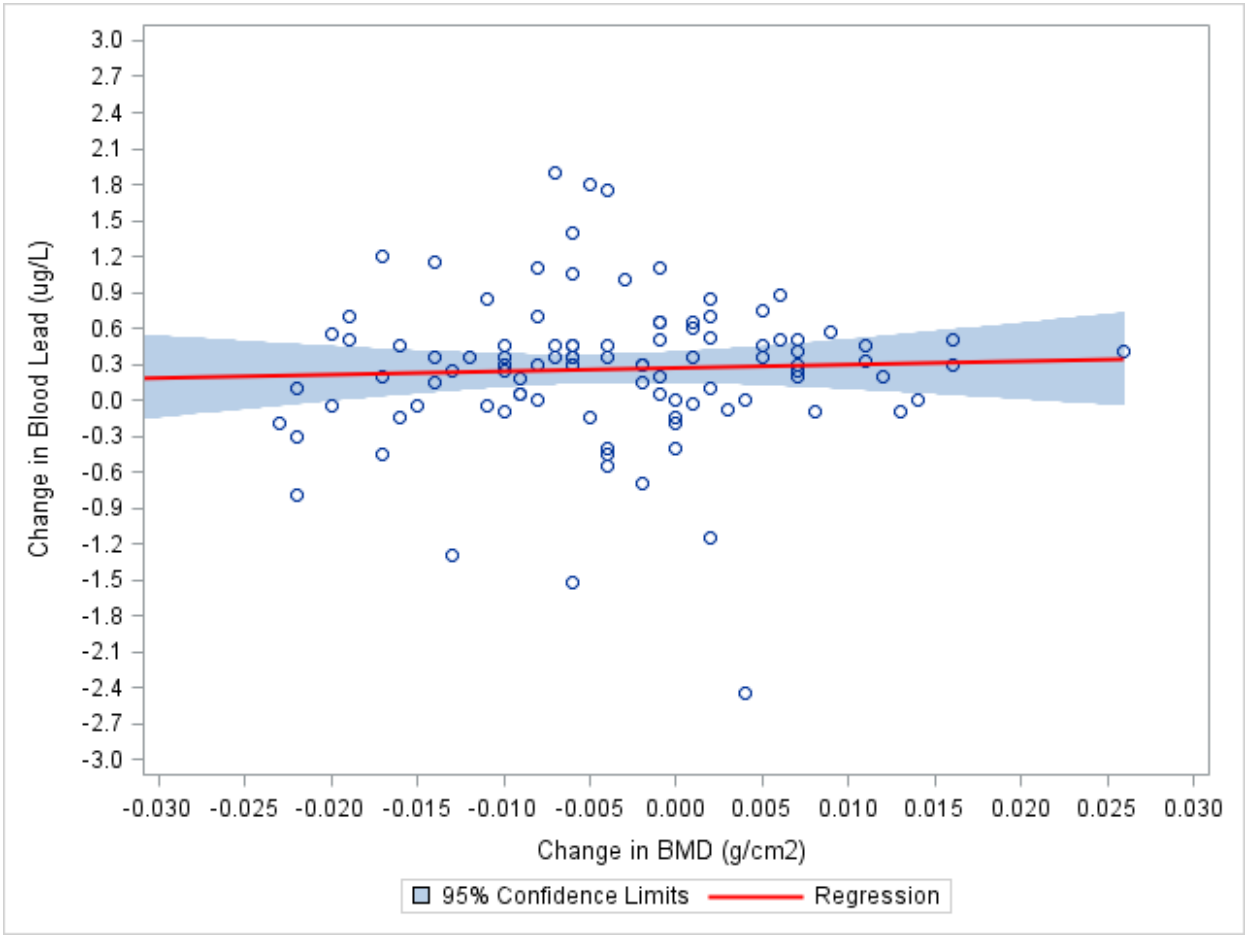
[c]Difference between second & first BMD

Table 3. Linear regression models assessing the association between change in maternal blood lead and change in BMD, age, race, education, smoking status, history of alcohol before pregnancy, BMI, current exercise, and calcium intake among pregnant women (n = 100)

Independent variables	Adjusted Estimated slope	95% CI
Intercept	-0.41	(-1.12, 0.30)
BMD change	-0.33	(-8.45, 7.80)
Age (years)*	0.02	(-0.00, 0.05)
Race (Black vs. White)	0.09	(-0.17, 0.34)
Education (<HS vs. ≥HS)	-	-
Smoking (Ever vs. Never)	-0.04	(-0.33, 0.24)
Drank alcohol before pregnancy (Yes vs. No)	-	-
Body mass index (kg/m^2)		
normal	ref	
overweight	0.03	(-0.28, 0.34)
obese	0.10	(-0.21, 0.40)
Current Exerciser	-	-
Calcium intake (mg/day)		
<1000	0.28	(-0.08, 0.64)
1000-<2000	ref	
≥2000	-0.01	(-0.30, 0.27)

FIGURES

Figure. Scatter plot of change in maternal blood lead values (µg/dL) vs. change in ultra-distal BMD g/cm2 among 100 pregnant women, Magee-Women's Hospital, Pittsburgh, Pennsylvania, 1992-1995. Each woman had both blood lead measurements from second and third trimesters and both BMD measurements that used the same arm.



APPENDICES

Appendix. IRB Letter of Exemption

EMORY UNIVERSITY | Institutional Review Board

TO: Heejoo Jo
Principal Investigator
Public Health

DATE: December 22, 2011

RE: **Expedited Approval**
IRB00053439
Bone Mineral Density and Blood Lead during Pregnancy

Thank you for submitting a new application for this protocol. This research is eligible for expedited review under 45 CFR.46.110 and/or 21 CFR 56.110 because it poses minimal risk and fits the regulatory category F5 as set forth in the Federal Register. The Emory IRB reviewed it by expedited process on 12/19/2011 and granted approval effective from **12/19/2011** through **12/18/2012**. Thereafter, continuation of human subjects research activities requires the submission of a renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above. A complete HIPAA waiver and a waiver of informed consent have been granted for this study.

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at www.irb.emory.edu, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, study design), you must submit an amendment request and secure IRB approval.

In future correspondence about this matter, please refer to the IRB file ID, name of the Principal Investigator, and study title.

Thank you,

Tom Penna, MTS
IRB Analyst Assistant
This letter has been digitally signed

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