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Impact of Bone Lead and Bone Resorption on Plasma and Whole Blood Lead Levels during Pregnancy

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The authors tested the hypotheses that maternal bone lead burden is associated with increasing maternal whole blood and plasma lead levels over the course of pregnancy and that this association is modified by rates of maternal bone resorption. A total of 193 Mexican women were evaluated (1997–1999) in the first, second, and third trimesters of pregnancy. Whole blood lead and plasma lead levels were measured in each trimester. Urine was analyzed for cross-linked *N*-telopeptides (NTx) of type I collagen, a biomarker of bone resorption. Patella and tibia lead levels were measured at 4 weeks postpartum. The relation between whole blood, plasma, and bone lead and NTx was assessed using mixed models. Plasma lead concentrations followed a U-shape, while NTx levels increased significantly during pregnancy. In a multivariate model, the authors observed a significant and positive interaction between NTx and bone lead when plasma lead was used as the outcome variable. Dietary calcium intake was inversely associated with plasma lead. Results for whole blood lead were similar but less pronounced. These results confirm previous evidence that bone resorption increases during pregnancy, with a consequential significant release of lead from bone, constituting an endogenous source of prenatal exposure. They also provide a rationale for testing strategies (e.g., nutritional supplementation with calcium) aimed at decreasing prenatal lead exposure.

bone resorption; lead; longitudinal studies; pregnancy

Abbreviations: BCE, bone collagen equivalents; NTx, cross-linked *N*-telopeptides.

Prenatal lead exposure, both from ongoing sources of maternal lead exposure and from the resorption of maternal bone containing long-lived stores of lead, has become an increasing source of concern as a risk factor for adverse reproductive outcomes and impaired fetal and infant development (1–9). Effective prevention of fetal lead exposure requires identification and control of sources of exposure for pregnant women. In an adult, 95 percent of lead accumulates in bone (10), with a half-life on the order of decades (11, 12). As a result, bone lead stores can remain elevated despite declines in environmental exposure. Pregnancy and lactation have been recognized as powerful stimuli for bone resorption (13–16), and there is concern that fetuses of women with high bone lead levels may be at risk for lead toxicity due to mobilization of bone lead stores.

Maternal calcium requirements increase in the early stages of pregnancy and continue to rise until delivery (17, 18). A full-term infant accumulates over 30 g of calcium during gestation, most of which is assimilated into the fetal skeleton in the third trimester. Maternal calcium needs are maintained by a fall in serum albumin concentration (19), increasing gastrointestinal absorption of calcium (20, 21), and an increase in bone resorption (13–16). Increased bone resorption during pregnancy has been of concern because of the potential transfer of bone lead into the fetal circulation via the maternal plasma compartment (22). Calcium is actively

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transferred to the fetus (23); maternal lead follows a transfer pattern similar to that of calcium and has no barrier at the placental level (22).

Mobilization of bone lead during pregnancy constitutes a major public health problem because of its potential impact on the developing fetal nervous system (24). Experimental and epidemiologic evidence strongly suggests that the developing fetus may be sensitive to disturbances from exposure to low concentrations of lead (22, 24).

Previous research suggesting that lead stored in the skeleton may become accessible during pregnancy has taken two different approaches. Gulson et al. (25–28) analyzed changes in blood lead isotope ratios in a cohort of pregnant women whose past lead exposure differed in lead isotopic composition from their current exposure. The other approach has relied on the study of longitudinal changes in whole blood lead concentrations (29–34). These studies have the limitation of not taking into consideration maternal bone lead concentrations, the intensity of bone resorption, and the relation between plasma and whole blood lead levels. While most (~99 percent) of the lead in blood is associated with erythrocytes, it has been suggested that the smaller fraction of lead in plasma may be the more biologically labile and toxicologically active fraction of circulating lead (35). Furthermore, there is evidence that the relation between plasma lead level and whole blood lead level is not as uniform as previously thought (36). Thus, the impact of lead remobilized from maternal bone stores may pass unnoticed if bone lead concentrations and direct measures of plasma lead are not taken into consideration.

The use of K X-ray fluorescence permits direct measurement of bone lead content. We have developed and validated an ultraclean method with which to accurately measure plasma lead levels and to incorporate both biomarkers in epidemiologic studies (36, 37). In this report, we present results from a longitudinal study in which we evaluated the hypotheses that bone lead burden and bone resorption are jointly associated with increasing plasma lead levels over the course of pregnancy.

MATERIALS AND METHODS

Study population

Between 1997 and 1999, our study personnel approached 462 potential participants at the Mexican Institute of Social Security in Mexico City, Mexico, providing them with a detailed explanation of the study. A total of 280 already-pregnant women were recruited; 182 women with a negative pregnancy test declared an intention to become pregnant in the near future and were also recruited. Of this group, 47 became pregnant, agreed to participate, and were enrolled in the cohort. This resulted in a study population of 327 subjects (figure 1). Exclusion criteria were: a pregnancy of more than 20 weeks' gestation; living outside of Mexico City; a physician's diagnosis of multiple pregnancy; a history of preeclampsia or pregnancy-related hypertension; psychiatric or cardiac disease; gestational diabetes; a history of repeated urinary tract infections; or seizure disorders requiring daily medication.

All women who agreed to participate were asked to visit our research center for a baseline evaluation and were scheduled for follow-up evaluations at 12, 24, and 34 weeks of gestation and at 1, 3, 7, and 12 months postpartum. During all visits, we administered a food frequency questionnaire and a physical examination that included anthropometric measurements and collection of urine (second void of the day) and blood samples. K X-ray fluorescence measurements of bone lead were performed only at the postpartum follow-up evaluations. Baseline evaluation also included a questionnaire assessing known risk factors for lead exposure (38). In this paper, we present only the results corresponding to the pregnancy evaluation, because the postpartum follow-up results were not yet available at the time of this writing.

The research protocol was approved by the human subjects committees of the National Institute of Public Health of Mexico, the Harvard School of Public Health, and the participating hospitals. All participants signed an informed consent form that included a detailed explanation of the study and the procedures used, as well as counseling on ways to minimize lead exposure.

Trained personnel performed all sample collections at the Center for Environmental Health Research of the American British Cowdray Medical Center in Mexico City. To minimize intraindividual variability and standardize plasma lead measurements, subjects were instructed to fast overnight prior to sample collection. An initial blood sample of 3 ml was collected in a low-lead container (Vacutainer B-D 367734; Becton-Dickinson, Franklin Lakes, New Jersey) for analysis of total lead. A second blood sample of 10–12 ml for plasma separation was collected via gravity-fed phlebotomy and was centrifuged at $800 \times g$ for 10 minutes at room temperature (37). All blood collections, plasma and whole blood processing, and sample analyses were conducted under high-efficiency particulate air (HEPA)-filtered conditions using trace metal clean techniques (39). Plasma lead levels were analyzed using a Finnigan element inductively coupled plasma high-resolution mass spectrometer (ICP-MS; Thermo Finnigan, Bermen, Germany).

Maternal bone lead measurements were obtained using a K X-ray fluorescence instrument (40). Thirty minutes of measurements were performed on the patella and midtibial shaft of each leg, representing trabecular and cortical bone, respectively. The two estimates for bone lead measurement (one for each leg) were computed, averaged, and weighted by the inverse of the proportion of the measurement error corresponding to each determination. To estimate maternal bone lead burden throughout pregnancy, we used the measurement obtained closest to the date of delivery. Eighty percent were obtained at 30 days postpartum; the average was 62 days.

Collection of urine samples was implemented several months after the cohort was assembled, so the number of participants was reduced to 193. This subgroup will be subsequently referred to as "participants in the NTx study" (figure 1).

We measured urinary excretion of cross-linked *N*-telopeptides (NTx) of type I collagen in a specimen collected at each visit. NTx is considered a specific marker of bone degradation that has been shown to be stable and resistant to degradation in

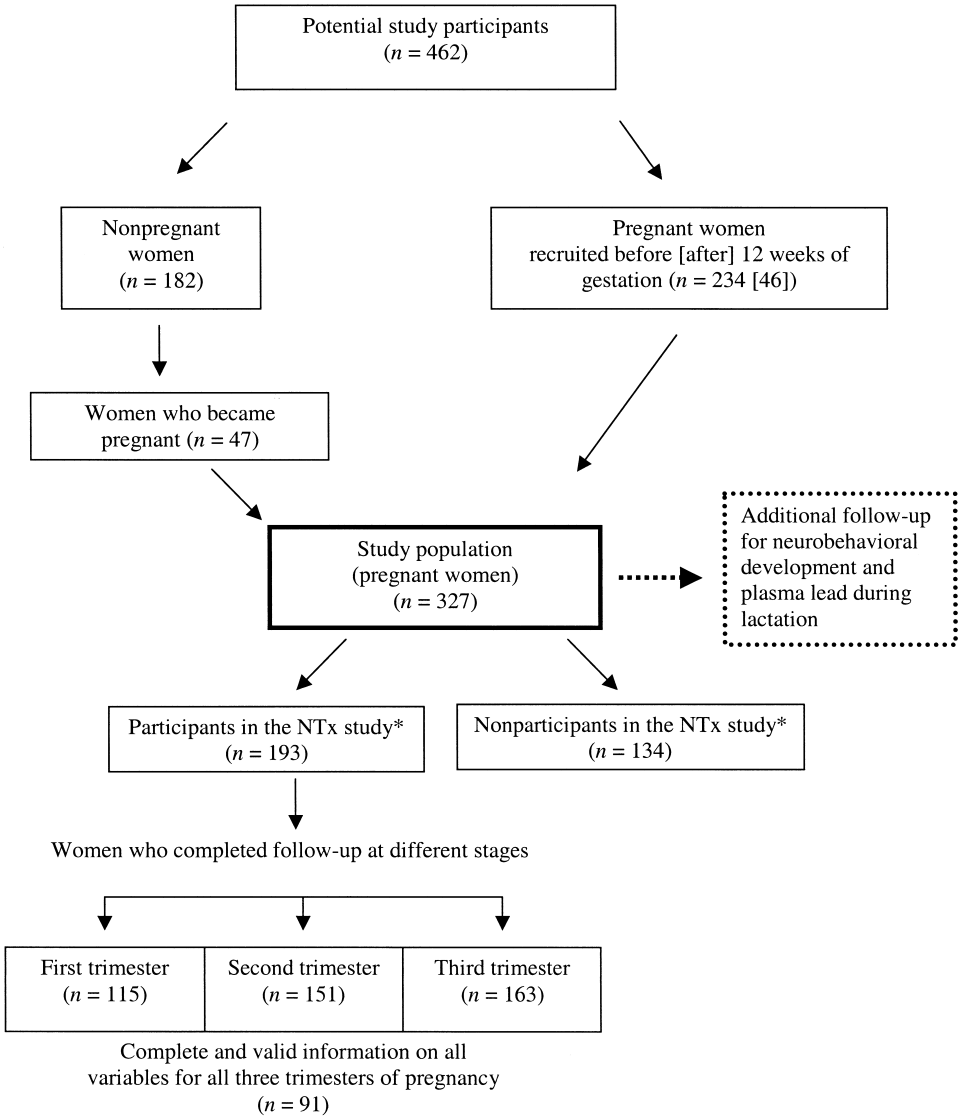

FIGURE 1. Selection of participants for a cohort study designed to assess mobilization of bone lead during pregnancy, Mexico City, Mexico, May 1997–April 1999. (*Participants/nonparticipants with/without urinary measurements of cross-linked *N*-telopeptides (NTx) of type I collagen.)

stored samples (41). Samples were analyzed with a commercially available competitive-inhibition enzyme-linked immunosorbent assay (41) (Osteomark; Ostex International, Seattle, Washington). NTx concentrations were expressed as nanomoles of bone collagen equivalents (BCE) per liter per millimole of creatinine per liter (nM BCE/mM creatinine).

Daily intakes of calcium and calories were assessed by means of a self-administered food frequency questionnaire developed and validated among women living in Mexico City, using the method of Willett et al. (42, 43).

Statistical analyses

The primary outcome variable was plasma lead level, measured longitudinally over the course of pregnancy. For the main exposure variable, we generated a composite variable (which served as an index of bone lead mobilization) equivalent to the product of bone lead concentration and urinary NTx level. The purpose of this index was to take into account the joint effect of bone lead burden (which is a fixed variable) and intensity of bone resorption (the time-varying variable NTx). To assess changes in the association between plasma and bone lead concentrations, we generated cross-sectional linear regression models for each trimester of pregnancy.

We then followed a longitudinal approach. We had to consider the fact that the observations were not independent in the analyses to avoid bias in the estimation of the standard errors. We used a mixed-effects modeling strategy (44), which takes this correlation into account, adjusts for independent variables that change over time, and accounts for

TABLE 1. Characteristics of participants and nonparticipants in a cohort study that assessed mobilization of bone lead levels during pregnancy, Mexico City, Mexico, May 1997–April 1999

Maternal characteristic	Participants (n = 193)			Nonparticipants (n = 134)			p value*
	Mean	SD†	Range	Mean	SD	Range	
Age (years)	27.10	5.48	15–43	26.39	4.98	17–41	0.25
Education (years)	10.58	2.94	2–18	10.77	3.56	0–24	0.79
No. of previous pregnancies	2.05	1.07	0–6	2.02	0.95	0–6	0.80
No. of children	0.81	0.89	0–4	0.79	0.74	0–3	0.85
Duration of residence in Mexico City (years)	24.08	8.15	0–43	23.38	8.22	0–40	0.40
No. of years of cooking with lead-glazed pottery	3.08	6.81	0–34	3.84	8.29	0–40	0.54
Initial blood lead level (μg/liter)	71.03‡	1.72§	15–328	68.19‡	1.75§	12–325	0.47
Patella lead level (μg/g bone mineral)	13.23	11.81	ND†–54.60	13.04	10.85	ND–37.68	0.27
	13.82¶	10.97¶		11.79¶	9.75¶		
Tibia lead level (μg/g bone mineral)	10.82	10.33	ND–37.07	9.12	14.32	ND–43.97	0.64
	11.35¶	8.82¶		13.71¶	9.17¶		

* Kruskal-Wallis equality of population rank test (62).
† SD, standard deviation; ND, nondetectable.
‡ Geometric mean.
§ Geometric standard deviation.
¶ Adjusted for negative values.

participants with missing data during follow-up. Only the intercept was treated as a random effect, and all of the independent variables were considered fixed effects. Since the plasma lead levels were not normally distributed, we logarithmically transformed the plasma lead values ($\log_e$). To compare the predictive capability of the bone lead mobilization index with regard to plasma lead versus blood lead, we also repeated the analysis using standardized values of $\log_e$-transformed plasma and blood lead concentrations. Standardization allows direct comparison of the estimated distribution-wise coefficients, which can be interpreted as mean change in the z score of either $\log_e$(plasma lead) or $\log_e$(blood lead) per one-unit increase in the NTx-bone lead product term.

K X-ray fluorescence provides a continuous, unbiased point estimate of the true bone lead measurement. However, negative values are sometimes produced when the true values are below the detection limit of the instrument (in this study, 8 percent and 11 percent of patella and tibia lead estimations, respectively, were below this limit). To avoid bias due to these negative values, we analyzed mean tibia and patella lead levels using both standard summary statistics that include the negative values and an estimation using interval regression, which simulates a normal distribution between 0 and the detection limit (45). To assess the potential influence of these negative values, we performed statistical analyses following two approaches: 1) including the negative estimates and 2) replacing them with new simulated values randomly generated with a uniform distribution between 0 and the lower limit of 2 μg lead/g bone mineral. Since the results obtained were very similar (~3 percent difference in the estimated coefficients), we present the results only from the former approach.

To rule out the possibility of potential bias due to loss of follow-up, we performed two separate analyses: one that included all women with at least one measurement during pregnancy (n = 193) and another that was confined to the subsample of women who completed follow-up and had no missing data (n = 91). Since we found no important differences with respect to estimated coefficients between these models, we decided to use the former approach. The results presented below refer to the 193 subjects who had at least one measurement taken during pregnancy. Statistical analyses were performed using Stata 8.0 (Stata Corporation, College Station, Texas) and S-Plus 6.2 (Insightful Corporation, Seattle, Washington).

RESULTS

The mean age of the participants was 27 years (range, 15–43 years). The participants had a mean duration of residence in Mexico City of 24 years. The use of lead-glazed ceramics was reported by 39 percent of the study population; 11 percent reported having eaten a meal cooked in this type of pottery on the day prior to an interview. We found no significant differences between women who participated in the NTx substudy and those who did not with respect to blood and bone lead levels, age, education, parity, number of abortions, duration of residence in Mexico City, and number of years of using lead-glazed ceramics (table 1).

The mean bone lead concentrations were 13.2 μg lead/g bone mineral and 10.8 μg lead/g bone mineral for patellar and tibial bone (13.8 μg lead/g bone mineral and 11.4 μg lead/g after correcting for negative values), respectively. We observed a statistically significant correlation (Pearson's ρ = 0.36; $p < 0.01$) between patellar and tibial lead levels; more-

TABLE 2. Characteristics of 193 pregnant women by trimester of pregnancy, Mexico City, Mexico, May 1997–April 1999

Maternal characteristic	Trimester of pregnancy						*p* value*
	First		Second		Third		
	Mean	SD†	Mean	SD	Mean	SD	
NTx† concentration (nM BCE†/mM creatinine)‡	75.97	38.86	101.48	48.91	143.74	60.52	<0.01
Blood lead level (μg/liter)	64.69§	1.68#	57.95§	1.65#	60.47§	1.65#	0.37
Plasma lead level (μg/liter)	0.13§	1.88#	0.12§	1.95#	0.12§	1.88#	0.51
Plasma:whole blood lead ratio (×100)	0.21	0.08	0.22	0.12	0.21	0.11	0.48
Daily calorie intake (kcal)	2,393.40	718.31	2,309.38	616.53	2,239.03	577.22	0.11
Daily calcium intake (mg)	989.22	393.85	1,043.50	360.63	1,000.09	331.44	0.55
No. of days during the previous week on which lead-glazed pottery was used for cooking	0.68	1.51	0.45	1.28	0.19	0.84	<0.01
Consumption of food cooked in lead-glazed pottery on the day before interview (%)	16.52		11.26		7.36		0.02

* Nonparametric test for trend across ordered groups (63).
† SD, standard deviation; NTx, cross-linked *N*-telopeptides [of type I collagen]; BCE, bone collagen equivalents.
‡ Nanomoles of bone collagen equivalents per liter per millimole of creatinine per liter.
§ Geometric mean.
# Geometric standard deviation.

over, the interaction terms patella-NTx and tibia-NTx, which we used as surrogates for the bone lead mobilization process, were even more correlated (Pearson's $\rho = 0.54$; $p < 0.01$). Thus, to avoid collinearity, we decided not to include the interaction terms simultaneously in the models presented.

We evaluated the potential contamination of plasma by lead from external sources (i.e., contamination by environmental lead during sample collection and processing) using lead concentration and stable isotopic composition analyses of procedural collection and processing blanks. We also evaluated potential contamination from internal sources (i.e., hemolysis of erythrocytes) using the presence of an elevated plasma hemoglobin level (>5 mg/dl) in combination with an elevated plasma lead:whole blood lead ratio (>0.5 percent). On the basis of these criteria, samples from four subjects were excluded.

Table 2 shows subject characteristics by trimester of pregnancy. Plasma and whole blood lead levels followed a U-shape during pregnancy. In contrast, urinary NTx concentrations showed a significant increasing trend. Plasma lead levels were highly correlated throughout the study period: The correlation from the first trimester to the second (Spearman's $\rho = 0.61$) increased further from the second trimester to the third (Spearman's $\rho = 0.71$). Daily intakes of calories and calcium showed no significant change. Subjects were advised to avoid using lead-glazed ceramics, which may explain the decreasing trend in the use of such ceramics (30 percent at baseline vs. 10 percent at the end of pregnancy).

A summary of plasma lead changes in relation to bone lead concentration and bone resorption intensity is shown in figure 2. Over the course of pregnancy, we observed an increasing trend for plasma lead concentrations among women with the highest bone lead burden (at or above the median level: 12.1 μg lead/g bone mineral); in contrast, we observed a decreasing trend among less-exposed women (below the median level). The observed increase reached its maximum among women with both the highest bone lead level and the highest bone resorption. In comparison with women with a low bone lead level and a high NTx level, those with a high bone lead level and a high NTx level had, on average, an 80 percent higher mean plasma lead level. These results support our original hypothesis of there being a biologic interaction between bone lead burden and bone resorption. They also suggest that as pregnancy progresses, bone lead may be mobilized increasingly into plasma.

In the cross-sectional analyses for each trimester of pregnancy, we also observed an increasingly stronger association between bone lead and plasma lead ($\log_e$-transformed) as pregnancy progressed. After adjusting for NTx, hematocrit, and use of lead-glazed ceramics in a linear regression model, we estimated that an increase in patella lead of 10 μg lead/g bone mineral would be associated with 9 percent ($p = 0.07$), 24 percent ($p < 0.01$), and 25 percent ($p < 0.01$) increases in plasma lead concentrations in the first, second, and third trimesters of pregnancy, respectively. The corresponding values for tibia lead were 8 percent ($p = 0.16$), 19 percent ($p < 0.01$), and 13 percent ($p = 0.01$), respectively.

Taking into account the observed increase in NTx concentrations as pregnancy progressed and the observation from the cross-sectional models that the strength of the bone lead-plasma lead association increased with length of gestation, we introduced an index of bone lead mobilization as an interaction term for the product of NTx and bone lead level in a longitudinal mixed-effects model. We followed this strategy to assess the joint effect of bone lead burden and bone resorption as pregnancy progressed. Since an association

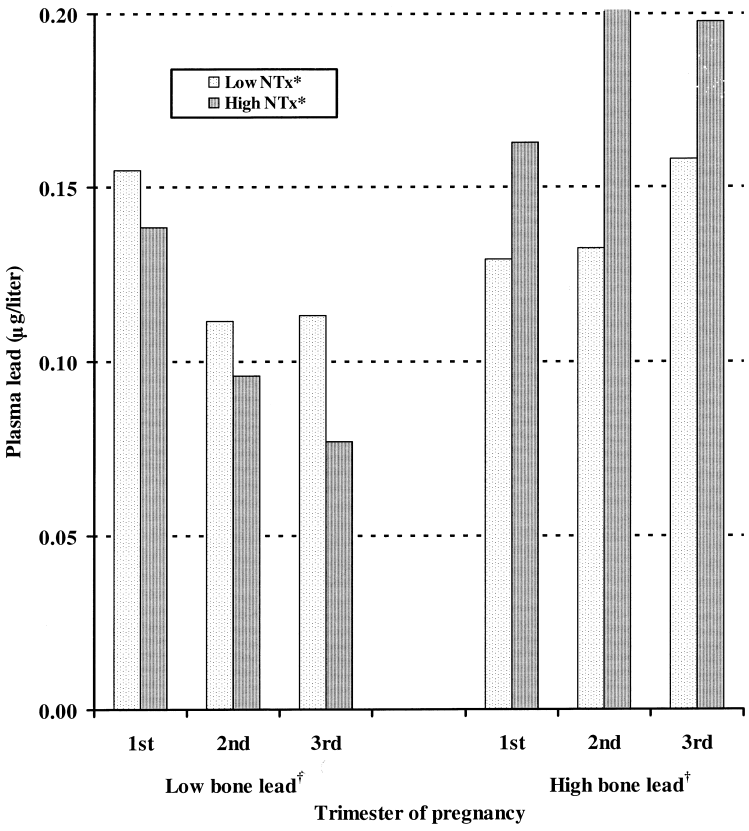

FIGURE 2. Mean plasma lead levels over the course of pregnancy, by bone lead concentration and a marker of bone resorption (cross-linked *N*-telopeptides (NTx) of type I collagen), Mexico City, Mexico, May 1997–April 1999. Women who had used lead-glazed ceramics the day before plasma sampling were excluded. (*Low NTx/High NTx: urinary NTx concentrations below/at or above the median value for that trimester. †Bone lead data were stratified according to the median value (12.1 mg lead/g bone mineral).)

between bone lead and plasma lead is only feasible in the presence of bone resorption, and bone resorption itself could (theoretically) only be associated with plasma lead levels if there was deposited bone lead to be mobilized, we decided not to include the main effects of either NTx or bone lead in the models presented.

We explored the potentially confounding effect of hemodilution (46), controlling for three proxy variables: hematocrit, percentage of maternal weight gained, and trimester of pregnancy. Neither hematocrit nor percentage of weight gained improved the fit of the model or changed the relation of interest. Trimester of pregnancy captured in a more integrated manner the physiologic changes that occurred over the course of pregnancy and was retained in the proposed model shown in table 3.

The coefficient of the bone mobilization index associated with trabecular bone was higher than the corresponding coefficient for cortical bone ($\beta = 0.010$ ($p < 0.01$) vs. $\beta = 0.007$ ($p < 0.01$)) for a 10-µg lead/g bone mineral increase in patella lead and tibia lead, respectively, and a 10 nM BCE/mM creatinine increase in NTx. The corresponding coefficients for the whole blood lead model were $\beta = 0.0065$ ($p < 0.01$) and $\beta = 0.0058$ ($p < 0.01$). Since these coefficients could not be directly compared, we employed the models using standardized values of $\log_e$(lead). Figure 3 shows the results. Standardized coefficients for the patella models were $\beta = 0.016$ (between-$R^2 = 0.1597$) and $\beta = 0.013$ (between-$R^2 = 0.1271$) for plasma and whole blood, respectively.

Calorie-adjusted calcium intake was inversely associated with plasma lead measurements, suggesting either decreased lead absorption in the gastrointestinal tract or decreased bone lead mobilization. The effect associated with a 100-mg daily calcium intake was higher if we restricted the model to women who reported eating foods cooked in lead-glazed ceramics ($\beta = -0.065$; $p = 0.04$) and to women with higher bone lead levels ($\beta = -0.040$; $p = 0.02$). We could not evaluate the interaction of these two factors because of the small number of women with high bone lead levels who reported eating from lead-glazed ceramics.

Figure 4 shows the relation between plasma lead and bone lead, adjusted for the covariates mentioned above. It graphically displays the results shown in table 3. Every curve represents the estimated relation between patella lead and plasma lead when NTx level is equal to its observed mean in each trimester of pregnancy.

TABLE 3. Results from a mixed-effects model of plasma lead levels (log_e-scaled) during pregnancy in a cohort of 193 women, Mexico City, Mexico, May 1997–April 1999

	Model for patella				Model for tibia			
	Regression coefficient	*t* value	*p* value	95% CI*	Regression coefficient	*t* value	*p* value	95% CI
Lead mobilization index†	0.010	5.12	<0.01	0.006, 0.014	0.007	2.90	<0.01	0.002, 0.012
Daily calcium intake (mg)‡	–0.026	–2.40	0.02	–0.046, –0.005	–0.024	–2.23	0.03	–0.046, –0.003
Consumption of food cooked in lead-glazed pottery on the day before interview (yes/no)	0.221	2.90	<0.01	0.072, 0.370	0.183	2.40	0.02	0.033, 0.333
Period of study§								
Second trimester	–0.175	–3.55	<0.01	–0.272, –0.078	–0.165	–3.32	<0.01	–0.262, –0.068
Third trimester	–0.216	–4.08	<0.01	–0.320, –0.112	–0.192	–3.53	<0.01	–0.299, –0.085

* CI, confidence interval; BCE, bone collagen equivalents.

† Estimated regression coefficient for a 10-μg lead/g bone mineral increase and a 10-nM BCE*/mM creatinine increase in cross-linked *N*-telopeptides of type I collagen.

‡ Estimated regression coefficient for a 10-mg increase. Calcium intake was adjusted for calorie intake.

§ Reference category: first trimester of pregnancy.

DISCUSSION

To our knowledge, this study was the first to evaluate longitudinal changes in plasma lead concentration during pregnancy as a function of bone lead burden and bone resorption (as measured by urinary NTx levels). We found a significant positive association between plasma lead concentration and the bone lead mobilization index. As the fitted model predicted, we observed that pregnant women with both higher bone lead burdens and higher bone resorption had greater plasma lead concentrations (figure 2). This suggests that their children may be at increased risk of prenatal lead exposure. This association was also observed with whole blood lead concentrations, although, as expected, the relation was not as strong as that observed in the plasma model. This is apparent in the models for the standardized biomarkers shown in figure 3.

Our data are consistent with the hypothesis that the skeleton is an important endogenous source of lead (11, 24). They indicate that endogenous sources of lead should be considered when exposure pathways and toxicity risk are

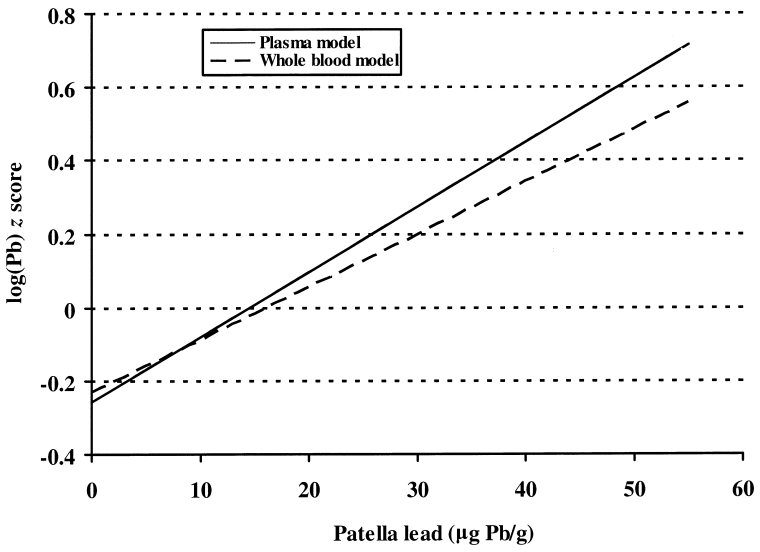

FIGURE 3. Comparison between the estimated associations of bone lead with plasma lead and whole blood lead when the urinary concentration of cross-linked *N*-telopeptides (NTx) of type I collagen equals the observed mean* (110 nM bone collagen equivalents/mM creatinine), Mexico City, Mexico, May 1997–April 1999. The curves show the adjusted† mixed-effects regression line for each model. Standardized log_e(lead) was fitted in both cases. (*The estimated association of bone lead with plasma lead varied as a function of NTx. †Results were adjusted for calcium intake, calorie intake, use of lead-glazed ceramics the day before sampling, and stage of pregnancy.) Pb, lead.

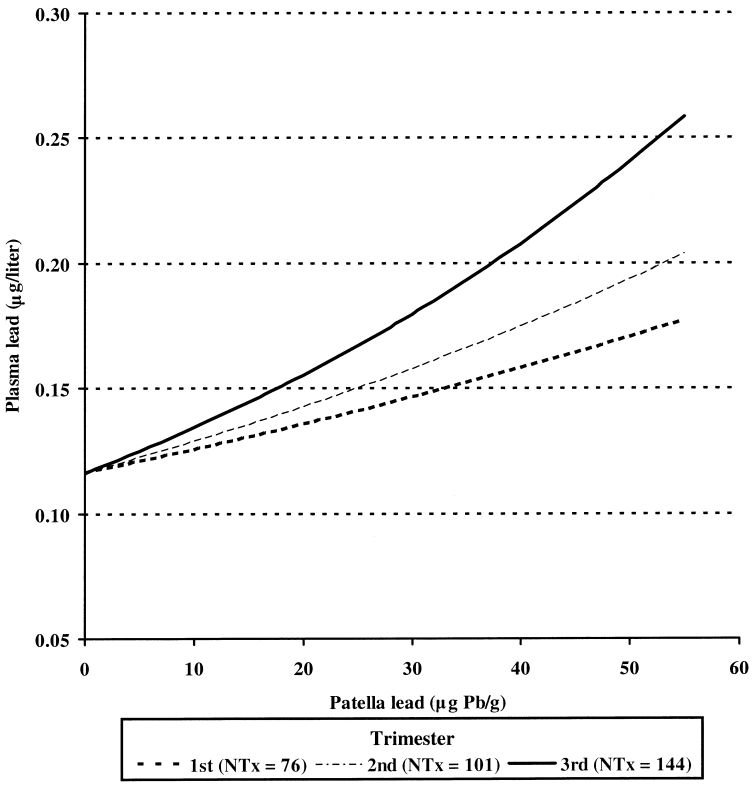

FIGURE 4. Mixed-effects model of the bone lead-plasma lead relation modified by bone resorption over the course of pregnancy, Mexico City, Mexico, May 1997–April 1999. The curves show the relation for three different urinary concentrations of cross-linked *N*-telopeptides (NTx) of type I collagen (adjusted for calcium intake, calorie intake, use of lead-glazed ceramics the day before sampling, and stage of pregnancy). Pb, lead.

being evaluated. Our data also suggest that increased calcium intake may decrease plasma lead levels, possibly because of the combined effect of decreased bone resorption and lowered gastrointestinal lead absorption.

During normal pregnancy, circulating concentrations of most nutrients decrease by the end of the first 10 weeks of gestation and remain lower than nonpregnant values until term (47). In contrast, most studies that have examined lead concentrations over the course of pregnancy have found that blood lead exhibits a U-shaped pattern during pregnancy, with a significant increase toward the end (29, 31–33, 48). Our results agree with this pattern (table 2). However, this trend may be apparent only when factors such as bone resorption and bone lead concentration are not taken into account. Our results suggest that the U-shape is less a reflection of what occurs in individuals than a consequence of the mixture of subjects with both low and high bone lead levels, a phenomenon we were able to distinguish by stratifying the data according to bone lead burden. Subjects with both lower bone lead and lower bone resorption showed a decreasing trend in plasma lead levels over the course of pregnancy. This observation suggests that in women with low lead burdens who experience lower bone mobilization, the small amount of lead released from bone is masked by the increase in plasma volume, thereby producing an apparent reduction in circulating lead levels. In contrast, women with both the highest bone lead burden and the highest bone resorption showed an increase in plasma lead level over the course of pregnancy, which suggests a large contribution of bone lead release—large enough to overcome the 45–50 percent increase in plasma volume (figure 2) (46). Blood lead levels, while not depicted in the figure, followed a very similar pattern. These last observations provide further evidence that maternal bone lead is an important source of prenatal lead exposure.

Calcium homeostasis undergoes changes during pregnancy to provide calcium for the fetus. This demand is met by enhanced maternal intestinal absorption and calcium mobilization from the skeleton. Few longitudinal reports on maternal bone mineral density have been published, and the results are conflicting. A minimal increase in bone mineral density during pregnancy was reported by Sowers et al. (49), while other investigators found either no change (21, 50) or a significant decrease (30, 51–53). Because of the potentially harmful effects of receiving radiation during pregnancy, most studies that have used dual-energy X-ray absorptiometry have measured women's bone mineral density before pregnancy and then repeated the measurement soon after delivery, missing the changes that occur during pregnancy.

Recent studies that have used ultrasonographic methods to measure bone mineral density have shown a progressive fall in bone mineral density over the course of pregnancy (15, 54–57). The estimated decrease in bone mineral density was approximately 6 percent of the early pregnancy value (57). The speed of sound in bone decreased in the second and third trimesters, though the decrease was greater in the third trimester. This observation is consistent with our finding of the highest NTx levels in the third trimester of pregnancy.

Several studies have documented increased bone resorption throughout pregnancy using different biomarkers (58–60). Values reaching 100–200 percent of nonpregnant values by the third trimester have been observed (14, 16). Our results agree with those of studies in which researchers conclude that bone resorption increases during gestation (13–16), as was suggested by the observed increase in NTx.

In recent studies, investigators who assessed longitudinal changes in blood lead levels during pregnancy have suggested that calcium intake may provide some protection against lead exposure (31, 34, 48). Hertz-Picciotto et al. (34) reported that a higher calcium intake was inversely associated with blood lead levels in the latter half of pregnancy. Our study provides support for this hypothesis; plasma lead levels were inversely related to dietary calcium intake. The mechanism by which calcium decreases plasma lead levels remains to be elucidated. A recent trial documented that calcium supplements decreased bone resorption in the last trimester of pregnancy (61). Similarly, studies that evaluated bone mineral density using ultrasonography have documented greater changes among women with a lower calcium intake (55). Further research in this area should focus on the evaluation of dietary interventions for decreasing prenatal lead exposure.

The main advantage of our study in comparison with previous reports is that we measured levels of plasma lead, which is believed to represent the active toxicologic fraction in blood. By studying bone lead concentrations and bone resorption simultaneously, we were able to examine their joint effect on plasma lead concentrations, taking into account several factors that might influence it.

In interpreting these results, one should take into account several methodological limitations. Information on dietary intake of lead was imperfect. In particular, we had data regarding the most important source of exposure—use of lead-glazed ceramics—but not on total lead intake. During pregnancy, calcium absorption increases (14); thus, women will also be likely to absorb more lead, since its absorption uses the same transport mechanisms as calcium. However, this increase in lead absorption does not explain the strong joint effect on plasma lead exerted by bone lead burden and bone resorption. In addition, we used an indicator variable for trimester of pregnancy, rather than a more appropriate variable like plasma albumin concentration, to account and control for the 45–50 percent increase in plasma volume estimated to occur during pregnancy (46). This may have prevented us from detecting increases in plasma lead concentration among women with lower bone lead and urinary NTx levels, and it may also have caused us to underestimate the plasma lead increases detected in women with higher bone lead levels.

Mobilization of bone lead from both the tibia and the patella was positively and significantly related to plasma lead levels, but the observed association was stronger for patella lead. The difference between the results obtained for the different anatomic sites could be attributable to the fact that the patella consists predominantly of trabecular bone, in which lead may be more available for mobilization because of increased mineral turnover rates compared with the cortical bone of the tibia.

In conclusion, our results suggest that, long after ongoing exposures have declined, past lead exposure still has the potential to affect maternal and fetal health through mobilization of bone lead stores. Our findings show that the increase in bone resorption during pregnancy may result in greater bone lead mobilization. Interventions focused on reducing the intensity of bone resorption during pregnancy could decrease prenatal exposure to lead.

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