Interrelations of Lead Levels in Bone, Venous Blood, and Umbilical Cord Blood with Exogenous Lead Exposure through Maternal Plasma Lead in Peripartum Women

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Recent research has raised the possibility that fetal lead exposure is not estimated adequately by measuring lead content in maternal whole blood lead because of the variable partitioning of lead in whole blood between plasma and red blood cells. Lead in maternal plasma may derive in large part from maternal bone lead stores. In this study we aimed to estimate the contribution of maternal whole blood lead, maternal bone lead levels, and environmental lead to umbilical cord blood lead levels (as a measure of fetal lead exposure). In the model, we assumed that lead from all of these sources reaches the fetus through the maternal plasma lead pathway. In 1994–1995, we recruited 615 pregnant women for a study of lead exposure and reproductive outcomes in Mexico City. We gathered maternal and umbilical cord blood samples within 12 hr of each infant's delivery and measured maternal lead levels in cortical bone and trabecular bone by a K-X-ray fluorescence (K-XRF) instrument within 1 month after delivery. We administered a questionnaire to assess use of lead-glazed ceramics (LGC) to cook food and we obtained data on regional air lead levels during the 2 months before delivery. We used structural equation models (SEMs) to estimate plasma lead as the unmeasured (latent) variable and to quantify the interrelations of plasma lead, the other lead biomarkers, and environmental lead exposure. In the SEM analysis, a model that allowed plasma lead to vary freely from whole blood lead explained the variance of cord blood lead (as reflected by a total model R^2; $R^2 = 0.79$) better than did a model without plasma lead ($r^2 = 0.67$). Cortical bone lead, trabecular bone lead, use of LGC, and mean air lead level contributed significantly to plasma lead. The exchange of lead between plasma and red blood cells was mostly in the direction of plasma to cells. According to the final model, an increase in trabecular bone lead and cortical bone lead was associated with increases in cord blood lead of 0.65 and 0.25 µg/dL, respectively. An increase of 0.1 µg/m³ in air lead was associated with an increase in the mean level of fetal cord blood lead by 0.67 µg/dL. With one additional day of LCG use per week in the peripartum period, the mean fetal blood lead level increased by 0.27 µg/dL. Our analyses suggested that maternal plasma lead varies independently from maternal whole blood lead and that the greatest influences on maternal plasma lead are maternal bone lead stores, air lead exposures, and recent cooking with LGC. The contributions from endogenous (bone) and exogenous (environmental) sources were relatively equal. Measurement of plasma and bone lead may be important in accurately assessing fetal lead exposure and its major sources, particularly if exogenous exposures decline. *Key words*: blood lead, bone lead, cord blood lead, maternal lead exposure, newborns, plasma lead. *Environ Health Perspect* 109:527–532 (2001). [Online 11 May 2001]
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Lead is a well-known human reproductive toxin. For centuries, lead exposure has been linked to adverse outcomes in pregnant women and newborns (*1*). In addition, fetal lead exposure can cause delay in the embryonic development of multiple organ systems, including retardation of cognitive development in early childhood (*2*).

Most studies that have used biomarkers to measure fetal lead exposure have relied on lead levels in maternal blood or umbilical cord blood at birth. However, recent research has cast doubt on whether either of these biomarkers reflects fetal lead exposure in a way that adequately predicts fetal lead toxicity. For example, in a recent epidemiologic study of women environmentally exposed to lead in Mexico City, maternal bone lead levels (but not maternal blood lead or umbilical cord blood lead levels) were related to declines in infant birth weight (*3*). In a companion study, maternal bone lead levels (but not maternal blood lead or umbilical cord blood lead levels) were related to smaller head circumference and shorter length at birth (*4*).

What parameters govern fetal lead exposure, and why is maternal bone lead the best predictor of fetal toxicity? Theoretically, fetal lead exposure must involve lead that is available to cross the placenta. Lead that is free to cross the placenta, in turn, must be derived from lead in plasma. Within the circulatory compartment, lead is partitioned primarily between red cells (> 99%) and plasma (< 1%). Because most lead is bound to red cells, whole blood lead levels (the biomarker most commonly measured) invariably reflect red-cell lead concentrations. However, the lead that segregates into red cells is bound and unavailable. If the < 1% of lead that is found in plasma were always in equilibrium with red-cell lead levels, whole blood lead would serve as an adequate proxy for plasma lead levels. However, recent research suggests that plasma lead levels can vary substantially without corresponding variations in whole blood lead levels (*5*). For example, Hernandez-Avila et al. (*6*) directly measured plasma lead among environmentally exposed adults in Mexico City and found that the plasma:whole blood lead ratio varied between 0.27% and 0.7%. In multivariate analyses, bone lead levels were found to influence plasma lead levels independent of the effect of whole blood lead levels. The strong influence of bone lead on plasma lead probably reflects the role of bone as the main repository (> 95%) of lead in adults (*5*). As further evidence of the importance of bone as a source of circulating lead, Gulson et al. (*7,8*) conducted a series of studies of women who had emigrated from Eastern Europe to

Australia. In these women, the lead stored in bone was a different isotopic ratio than lead in their current environments. The investigators demonstrated that pregnancy and lactation were associated with large increases in the amounts of lead released from bone.

Together, these findings suggest that fetal lead exposure, reflected by plasma lead levels, may not be represented adequately by maternal whole blood lead levels and that the importance of bone lead levels as an independent determinant of fetal lead toxicity may be explained by the importance of bone lead as a source of circulating lead and by its influence on lead liberated into plasma.

What factors influence plasma lead levels during pregnancy, and why not simply measure plasma lead levels? Unfortunately, direct measurement of lead in plasma is an extraordinarily challenging procedure because of several factors. Among the most important are the potential for plasma lead to be significantly contaminated by lead in red blood cells with even a small amount of hemolysis; the extremely low levels of lead in plasma (< 0.1 µg/dL), which can be measured precisely and accurately but requires special instruments; and the potential for environmental contamination from equipment and from other sources during phlebotomy, specimen transports, digestion procedures, and analysis.

As a consequence, few studies have investigated plasma lead levels, and, to the best of our knowledge, none has done so during pregnancy. Because umbilical cord blood lead levels are the best available measure of fetal lead exposure and because plasma lead must be the vehicle for both exogenous (environmental) and endogenous (bone) sources of lead that influence fetal lead exposure, the role of plasma lead in fetal lead exposure can be investigated directly by examining interrelationships between existing biomarkers of lead exposure and assuming that cord blood lead is influenced most by plasma lead.

The aim of the current study was to use data from a large epidemiologic study ($n > 600$) of lead and reproduction and to apply structural equation models (SEMs) to estimate the effects of endogenous and exogenous lead exposure on fetal cord blood lead through maternal plasma lead. The women who participated in this study live in Mexico City, where environmental lead exposure and consequently bone lead levels have been high (9). Measurements were available for environmental sources of lead exposure and levels of lead in maternal whole blood, maternal trabecular and cortical bone, and umbilical cord blood.

Materials and Methods

Study subjects. From 28 April 1994 to 30 June 1995, women attending any of three hospitals (Mexican Social Security Institute, Manuel Gea Gonzalez Hospital, or National Institute of Perinatology) for prepartum examination in Mexico City were recruited. Interviewers explained the study in detail and obtained informed consent from participants. The study protocol was approved by the Human Subjects Committee of the National Institute of Public Health (Mexico City, Mexico). Women were excluded if they had a physician's diagnosis of multiple fetuses, preeclampsia, psychiatric, kidney or cardiac diseases, gestational diabetes, history of repeated urinary infections, seizure disorder requiring daily medication, ingestion of corticoids, systolic blood pressure > 140 mm Hg, or diastolic blood pressure > 90 mm Hg.

Maternal and umbilical cord blood lead test. Maternal and umbilical cord blood samples were gathered within 12 hr of each infant's delivery. Blood samples were analyzed by atomic absorption spectrometry (Perkin-Elmer 3000; PerkinElmer, Norwalk, CT, USA) at the metal laboratory of the American British Cowdray Hospital in Mexico City. Analyses of external blinded quality-control samples were provided throughout the study period by the Wisconsin State Laboratory of Hygiene (WSLH) Cooperative Blood Lead Proficiency Testing Program (PBPTP) (Madison, WI, USA). On tests of blinded specimens in the ranges of 0–8, 14–22, and 40–50 µg/dL, the laboratory performed with accuracy within 5.0% and a coefficient of variation (CV) < 15%, which demonstrated good precision and accuracy of the determinations made at American British Cowdray Hospital.

Bone lead test. At each mother's visit to the research center at American British Cowdray Hospital 1 month after delivery, maternal bone lead levels were measured on each woman's mid-tibia shaft (cortical) and patella (trabecular) with a spot-source ^{109}Cd K-XRF instrument (ABIOMED Inc., Danvers, MA, USA). Thirty-minute measurements were taken at each bone site after the skin had been washed with 50% isopropyl alcohol. The K-XRF beam collimator was sited perpendicular to the bone surface for the tibia and 30° in the lateral direction for the patella. The introduction of ^{109}Cd as an excitation γ-ray source, with 88 keV photons emitted from approximately 4% of its decays, provokes the emission of fluorescent photons from the target. The resulting signal sensed by the detector, high-purified germanium crystal, undergoes processing by the amplifier-computer system for subsequent display and analysis. The detection system collects, counts, and analyzes the emitted X-rays over the wavelength spectrum, from which the net lead signal is determined after subtraction of Compton background counts,

by a linear least-square algorithm. The lead fluorescence signal is then normalized to the elastic or coherently scattered γ-ray signal, which arises predominantly from the calcium and phosphorus present in bone mineral. The unit of measurement is micrograms per gram (µg/g) of bone mineral (10). Because the K-XRF instrument provides a continuous, unbiased estimate that oscillates around the true bone lead value, negative value estimates are sometimes produced when true bone lead values are close to zero. The instrument also provides an estimate of uncertainty association with each measurement, which is derived from a goodness-of-fit calculation of the spectrum curve and is equivalent to a single standard deviation. Although a minimal detectable limit calculation of twice this value has been proposed for interpreting an individual's bone lead estimates, statistical experiments have shown that retention of all point estimates makes better use of the data in epidemiologic studies (11,12). Validation studies of the instrument indicated a high degree of precision and accuracy of point estimate and uncertainty measurement in comparison with chemical analysis in studies of lead-doped phantoms (13).

Use of lead-glazed ceramics. Previous studies have shown that use of low-temperature–fired lead-glazed ceramics (LGC) is a major source of lead exposure in Mexico City (15,19). To evaluate this exposure, we interviewed participants in the research office by trained personnel using a structured questionnaire. Using pictures to help identify these LGC, we asked participants about the frequency of LGC use in the previous week (number of days used per week) and about the number of years they had used LGC to store and cook food. The product of these two variables was used to estimate the total lifetime days of LGC use:

Total lifetime days of LGC use =

$$\frac{365.25}{7} \times (\text{days of use per week}) \times (\text{years of use})$$

Air lead concentrations. We used the air lead data supplied by the Environmental Air Monitoring Network of Mexico City. Nineteen monitoring points in Mexico City provide information on air lead levels, which were measured four to five times per month. We used information regarding the area of residency of participants and assigned these data to subjects depending on their area of residence and the date of delivery. The mean of air lead levels for the previous 2 months before delivery was the index of lead exposure from ambient air for each woman. In addition, we used the number of years of residence in Mexico City to indicate chronic exposure.

Estimated lead in red blood cells.
Because < 1% of lead is present in plasma (*6,16*), we used whole blood lead level divided by hematocrit (BPb/Hct) to estimate lead concentration in red blood cells (RBC). Out calculation is:

$$\text{Pb Conc in RBC} = \frac{\text{Lead in RBC}}{\text{RBC volume}}$$
$$= \frac{(\text{Lead in whole blood}) \times 99\%}{\text{Whole blood volume} \times \text{Hct}}$$
$$= \frac{(\text{Lead in whole blood})}{(\text{Whole blood volume})} \times \frac{1}{\text{Hct}}$$
$$= \frac{(\text{Lead conc in whole blood})}{\text{Hct}}$$

Data analysis. Descriptive statistics. We examined univariate distributions of continuous variables to determine the normality of the variables. To better attain normal distributions, maternal and cord blood lead values were natural log-transformed. Outliers were identified using the extreme studentized deviate (ESD) procedure (*17*). Percentiles for the procedure are approximated on the basis of the *t*-distribution (*18,19*). To assess the potential influence of outliers, we repeated our SEM analyses after excluding four outliers. We used Pearson correlation analyses to determine the intercorrelations among the lead biomarkers (maternal whole blood and bone lead levels, umbilical cord blood lead level) and use of LGC, air lead levels, maternal age, and time of residence in Mexico City in both years and percentage of life span spent in Mexico City (years of residence in Mexico City divided by age).

Multiple regression analyses. As predictors of fetal cord blood lead levels, the independent influence of age, our maternal lead biomarkers, and our external exposure parameters, including use of LGC, air lead levels, and percentage of life span in Mexico City, were examined.

Structural equation models. SEMs were used to express the relationships among variables, including measured variables and plasma lead (latent variable) (*20*), and thus to test the hypothesis that maternal bone lead levels contribute to fetal lead exposure independent of maternal whole blood lead levels. Structural equation modeling allows one to estimate simultaneously a set of interrelated regression models. For example, bone lead must be considered an outcome in one regression (depending on use of LGC, length of residence in Mexico City, and the like). In a second regression model it is a predictor of umbilical cord lead. By combining these into one system of equations, one gains several advantages. First, estimates may be made more precisely, taking into account the covariance structure of all of the variables. Second, downward bias that results from putting variables on the casual pathway into

the regression (*21*) may be avoided. Third, the system of equations may be compactly written in terms of a path diagram that illustrates the multiple relationships. Finally, unmeasured (latent) variables may be included when there is sufficient ancillary information. While this produces noisy estimates of the latent variable, recognizing its existence usually increases the precision of the estimates of the associations among the measured variables. These models have been well developed and described since the 1960s. Our analysis approach conceptualizes plasma lead levels as the unmeasured, "latent" variable through which lead levels in the maternal patella, tibia, and venous blood can influence umbilical cord blood lead levels (as fetal lead exposure).

To fit our model, we established the network of causal relationships among the variables (Figure 1). The direction of causality among these variables is clear. All that is unknown is the magnitude of the associations (which for some pathways may be zero). As a second step, we constructed a structural model with a latent variable plasma lead level added. This allowed us to test the hypothesis that such a model fit the data better and that some variables may influence cord lead through plasma rather than erythrocyte lead. We used the covariance analysis of linear structural (CALIS) equation in SAS package 6.12 (CALIS procedure; SAS Institute Inc., Cary, NC) for SEM analysis (*22*). All these analyses used the maximum likelihood method of parameter estimation, and all were performed on the variance–covariance matrix (*20*).

We assumed that missing data occurred completely at random (MCAR). Missing data were the result of incomplete questionnaires and incomplete collection of blood samples. The most common reasons for failure to collect blood samples and questionnaires were inconvenience and the time-consuming

nature of the interview process. In the data analysis, we used the pairwise deletion method to treat missing data; each correlation and covariance between two variables was computed from pairwise-complete data, with the exclusion of cases that had missing values for one or both variables (*23,24*).

Results

A total of 615 pregnant women had bone lead measurements. The most common reason for which women did not have bone lead measurements were residence outside Mexico City and the length of time required for the measurement. Table 1 shows the characteristics of study subjects. Bone lead levels were considered missing if they were associated with excessive measurement uncertainty (> 15 μg/g in patella and > 10 μg/g in tibia) reflecting quality assurance procedures used previously (*11,25*). Thus, only 575 women had patella lead levels and 603 had tibia lead levels; the mean (SD) values for the patella and tibia lead values were 14.24 (14.19) and 9.67 (9.21) μg/g bone mineral, respectively, and 86 (14%) and 78 (13%) of these values, respectively, were 0 or negative, signifying very low levels measured with error. Among the women who used LGC for cooking, 25% reported using LGC ≤ 3 day per week and 8% reported use ≥ 3 days per week (mean 2.92 days per week, SD = 2.28). About 20% of the women had used LGC for ≤ 5 years and 16% of the women for ≥ 5 years (mean 9.03 years, SD = 9.13). The mean number of days of lifetime LGC use, calculated from the above variables, was 1,489 (SD = 2,010). Twenty-six women (4%) reported smoking during pregnancy; about 39% had smoked in the past but not during pregnancy.

Table 2 shows the Pearson correlation coefficient matrix. Natural log-transformed cord blood level was significantly correlated with all maternal lead biomarkers, use of
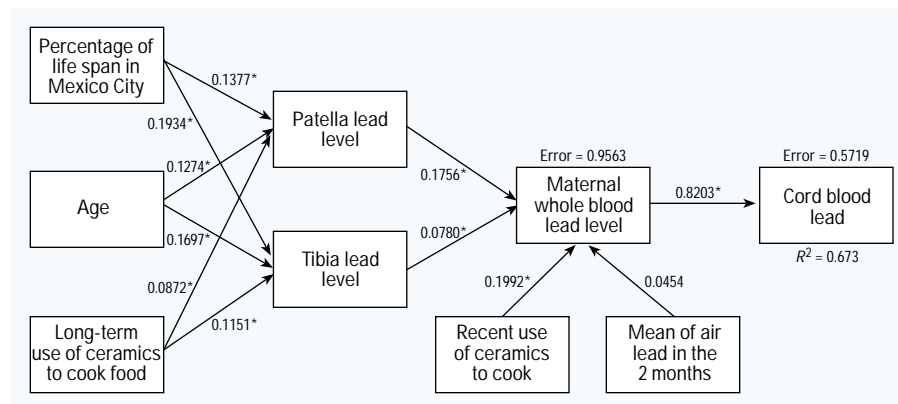
Figure 1. Manifest model of interrelationships of bone lead and umbilical cord blood lead in peripartum women. Standardized coefficients are presented.
*p < 0.05.

LGC, air lead level, and years of residence in Mexico City. Number of years living in Mexico City was significantly correlated with age (R^2 coefficient = 0.45). To avoid collinearity between these two variables, we used percentage of life span spent in Mexico City, which did not correlate with age (R^2 coefficient = –0.057, p = 0.159). Few women reported smoking while pregnant (n = 26). Neither current nor past smoking was associated with any of the lead biomarkers. Thus, we did not include smoking status in further analyses.

In a multiple regression analysis (data not shown), maternal blood lead level was highly associated with fetal cord blood lead level. Maternal tibia lead level, patella lead level, and the other exposure indices (including LGC use, air lead level, and percentage

of life span in Mexico City) were not significantly associated with cord blood lead levels. However, as discussed by Breslow and Day (*21*), this regression analysis was inappropriate because maternal blood lead was an intermediate marker for sources of lead exposure, (i.e., maternal blood lead level was influenced by bone lead and external sources of lead exposure). Failure to account for these interrelationships weakened the analysis. Thus, we used SEMs to assess the interrelationships of these factors.

Figures 1 and 2 depict the results obtained in SEMs without and with the plasma lead level respectively. The coefficients in these two figures were standardized to represent a 1 SD change in each exposure pathway. In testing for overall model fits, we found the goodness-of-fit index (GFI) and

degree-of-freedom–adjusted GFI in both models were ≥ 0.9 (1 = perfect fit). In both models, Bentler's comparative fit indices were ≥ 0.95 (1 = perfect fit). All indices for model fit revealed an acceptable fit in both models (*20*). The SEM used for the analysis shown in Figure 1 revealed that both cortical and trabecular bone lead levels significantly contributed lead to the maternal blood pool in pregnant women. The lead in patella (trabecular bone) contributed more than that in tibia (cortical bone). Long-term use and recent use of LGC were positively correlated with bone and blood lead levels, respectively. However, air lead levels measured 2 months before delivery were only weakly associated with whole blood lead in the model. Overall, the coefficient of maternal whole blood lead to cord blood lead was similar to the regression coefficient and explained about 67% of variance in the cord blood lead level.

In the model used for the analysis in Figure 2, we implanted a latent variable, plasma lead, and RBC lead to represent two components in the blood pool. First, we observed that both bone lead measures contributed significantly to maternal plasma lead. Second, we found that recent air lead level was significantly associated with plasma lead but not with whole blood lead. To compare the coefficients in the different models, we standardized them to represent a 1 SD change in each blood index. The standardized coefficient for whole blood lead level was 0.8203 whereas that for plasma lead level was 0.8897. On the other hand, the R^2 values were 0.792 in the plasma model and 0.673 in the whole blood model. Both results suggest that plasma lead affects fetal cord blood lead stronger than maternal whole blood lead level.

Table 3 shows estimates of fetal cord blood changes, calculated from the final SEM in Figure 2 with use of nonstandardized coefficients (original or natural coefficients) and

Table 1. Characteristics of study subjects.

Variable	No. subjects[a]	Mean (SD) or %
Maternal blood lead, µg/dL	608	8.45 (3.94)
Maternal hematocrit	604	0.41 (0.04)
Estimated RBC lead	597	20.59 (9.54)
Umbilical cord blood lead, µg/dL	509	6.55 (3.45)
Patella lead, µg/g bone mineral	575	14.24 (14.19)
Tibia lead, µg/g bone mineral	603	9.67 (9.21)
Maternal age, year	614	24.52 (5.11)
Residence in Mexico City, year	613	20.57 (8.23)
Residence in Mexico City, % of life span	613	84.22 (29.55)
Air lead 2 months before delivery, µg/m³	522	0.21 (0.02)
Current smoker	26	4.2%
Ex-smoker	242	39.3%
Cooking in LGC in the week before delivery		
No	406	66.0%
1–3 days/week	154	25.0%
4–7 days/week	49	8.0%
Unknown	6	1.0%
Cooking in LGC in lifetime		
No	354	57.6%
≤ 5 years	122	19.8%
6–10 years	36	5.9%
11–15 years	8	1.3%
> 15 years	53	8.6%
Unknown	42	6.8%

[a]The numbers are not consistent because of missing data.

Table 2. Correlation coefficient matrix for lead biomarkers, use of LGC, age, residence in Mexico City, and smoking status.

	Log MPB	Pat	Tibia	Age	Cook	PbAir	Time Mex	P_Y Mex	Day	Year	Smoke
LogCPB	0.82	0.23	0.18	0.08	0.15	0.10	0.12	0.07	0.21	0.20	0.05
p-Value	0.0001	0.0001	0.0001	0.0638	0.0011	0.0404	0.0072	0.1045	0.0001	0.0001	0.2601
n	506	472	498	508	468	440	507	507	503	471	508
LogMPB		0.2232	0.1493	0.0473	0.1864	0.0417	0.1290	0.1184	0.2336	0.2167	0.02866
p-Value		0.0001	0.0003	0.2451	0.0001	0.3431	0.0015	0.0035	0.0001	0.0001	0.4810
n		569	596	607	565	518	606	606	602	568	607
Pat			0.2630	0.1240	0.0910	0.0082	0.1830	0.1287	0.1420	0.0702	0.0659
p-Value			0.0001	0.0029	0.0360	0.8571	0.0001	0.0020	0.0007	0.1052	0.1148
n			564	574	531	488	573	573	569	534	574
Tibia				0.1645	0.1200	–0.0550	0.2441	0.1816	0.1444	0.1129	0.0593
p-Value				0.0001	0.0045	0.2127	0.0001	0.0001	0.0004	0.0075	0.1461
n				602	558	515	601	601	597	561	602
Age					0.0506	–0.0913	0.4547	–0.0569	0.0241	0.0861	0.04342
p-Value					0.2280	0.0373	0.0001	0.1594	0.5526	0.0393	0.2828
n					570	521	613	613	609	573	614

Abbreviations: LogCPB, natural log-transformed cord blood lead; LogMPB, natural log-transformed maternal blood lead; Pat, patella lead; Tibia, tibia lead; Cook, total lifetime days of cooking with lead-glazed ceramics; PbAir, mean of air lead 2 months before delivery; TimeMex, years living in Mexico City; P_Y Mex, percentage of life span in Mexico City; Day, days of cooking with lead-glazed ceramics per week; Year, years of cooking with lead-glazed ceramics in lifetime; Smoke, smoking or not during pregnancy.

an antilogarithmic calculation method (cord blood lead level, maternal blood lead level, and RBC lead level were log-transformed in the models). According to these estimates, an increase of 1 µg/g in maternal patella lead was associated with an increase in mean level of fetal cord blood lead by 0.032 µg/dL. An increase in maternal patella lead from the first quartile (4.26 µg/g) to the third quartile (23.71 µg/g) was associated with an increase in mean level of cord blood lead by 0.65 µg/dL. The contribution from tibia (cortical bone) was a little less than that from patella (trabecular bone). An interquartile range change in both bone lead measures was associated with an increase in cord blood lead by about 1 µg/dL. One additional day of LGC use per week in the peripartum period was associated with an increase in mean level of fetal blood lead by 0.27 µg/dL. Fetal blood lead was also influenced by air lead levels in peripartum period: Each 0.1 µg/m^3 change in air lead was associated with a 0.67 µg/dL change in mean level of fetal cord blood lead. As a plausibility check on this model, we note that Crocetti et al. (26) showed that gasoline use contributed to about 9 µg/dL of blood lead levels in U.S. children in 1976, a time when air lead concentrations in U.S. urban areas were typically 1–1.25 µg/m^3, a result quite consistent with the air lead–blood lead ratio we see.

Discussion

Our study suggests that incorporating plasma lead as a latent variable in models used to analyze the relationship of bone lead and exogenous lead exposure with cord blood lead reveals important relationships. In our analysis with SEMs, maternal plasma lead level had a stronger association (standardized coefficient = 0.8897) than that of whole blood lead (standardized coefficient = 0.8203) to fetal cord blood lead level, and the fit of the model that incorporated plasma lead (R^2 = 79%; Figure 2) was substantially better than the fit of the model that omitted plasma lead (R^2 = 67%; Figure 1). Moreover, the model demonstrated a significant contribution of lead from the skeletal system to plasma during pregnancy, a contribution that is independent of the influence of maternal RBC lead. One can infer that plasma lead is an important biomarker for fetal lead exposure and that maternal whole blood lead levels do not accurately capture the influence of bone lead on fetal lead exposure. Use of a biologically plausible model for the pathways of lead kinetics greatly increased our ability to separate out the contributions from different sources. For example, lead in both cortical and trabecular bones was released into blood during pregnancy. In a standard multiple regression analysis, we cannot easily separate the contribution from each pathway to maternal blood lead level and study their effects on fetal cord blood lead by way of maternal blood or plasma lead levels because of correlation and collinearity between patella and tibia lead levels.

Four sources of lead contributed significantly to plasma lead. Recent use of LGC and lead in trabecular bone (patella) had the largest and the second largest standardized coefficients, respectively—results indicating that these two sources had the greatest influence on plasma lead levels. The greater contribution of trabecular than of cortical bone probably reflects the greater vascularity and higher turnover rate of trabecular bones. Use of LGC, a documented source of lead exposure in women and children in Mexico City (15), was a source of food contamination and a path of lead ingestion in this study; lead absorbed by the gastrointestinal tract is quickly detectable in the plasma. The standardized coefficient for air lead level was the smallest of these four pathways, but this finding was in part a reflection of the relatively narrow distribution of air lead levels to which these women were exposed, which, in turn, is a reflection of the effort in Mexico over the last 10 years to phase out the use of leaded gasoline. Using original coefficients (normal scale) of SEM in Figure 2, a change of 0.1 µg/m^3 in air lead level would have a sizable effect on fetal cord blood lead level (0.67 µg/dL).

The effect of RBC lead on plasma lead detected in our model revealed that most lead moved from plasma to RBC and less from RBC to plasma. The coefficient ratio of lead movement into/out of RBC was about 6. The kinetic equilibrium between RBC lead and plasma lead has been studied previously (7,27,28). Both Marcus (27) and deSilva (28) demonstrated a nonlinear relationship between plasma lead and blood lead with a kinetic transfer ratio (into/out of RBC) ranging from 214 to 373. Plots in these studies revealed a J-shaped curve for plasma versus blood lead. However, their analyses were on a normal scale, while ours used natural logarithm-transformed RBC and cord blood lead levels. Exponentiation of our ratio gives a value of 403, which is similar to the results obtained by their investigations. Because we log-transformed both variables, the shape of the relationship in our model followed a power law and incorporated much of the nonlinearity in the models used by Marcus and deSilva.

Recent studies with a lead isotope composition method have proved that maternal skeletal lead is mobilized during pregnancy, makes major contributions to the maternal blood pool, and is transferred to the fetus (7,8). In these studies, a higher $^{206}Pb/^{204}Pb$ ratio reflected the abundance of the ^{206}Pb
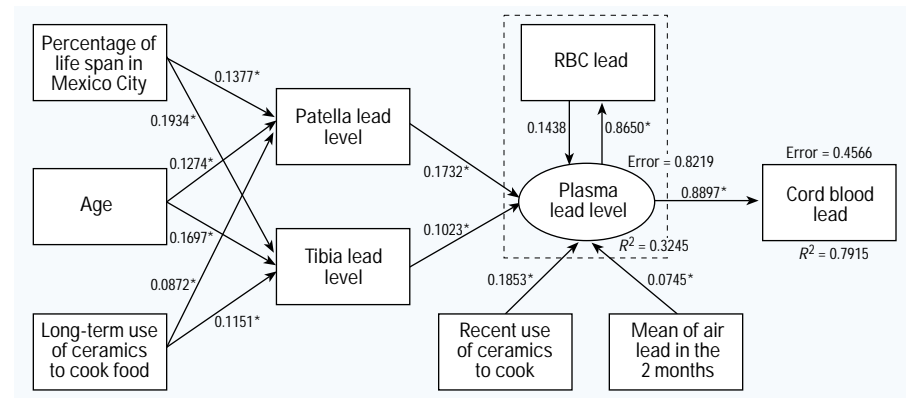
Figure 2. Latent model of interrelationships of bone lead and umbilical cord blood lead in peripartum women to estimate the effect of plasma lead. Standardized coefficients are presented.
*p < 0.05.

Table 3. Changes in cord blood lead level associated with specified changes in endogenous and environmental factors, according to the final structural equation model with normal scale unit.

Variables	Cord blood lead change (µg/dL)
Patella lead change	
1 µg/g of bone mineral	0.032
Interquartile (4.26–23.71 µg/g of bone mineral)	0.65
Tibia lead change	
1 µg/g of bone mineral	0.022
Interquartile (4.08–15.40 µg/g of bone mineral)	0.25
Cooking with LGC	
1 day/week	0.27
Interquartile (1–3 days/week)	0.56
Air lead level change	
0.1 µg/m^3	0.67

isotope in the country where the women grew up and was different from the isotopic ratio (lower) in their current country of residence. The increased isotopic ratio in blood when these women were pregnant reflected the bone lead (with higher isotopic ratio) released into blood. The skeletal contribution of lead to blood lead level, based on Gulson's (*30*) study of nine pregnant immigrant women, produced a mean increase of 31% in the $^{206}Pb/^{204}Pb$ ratio (range, 9–65%). These studies provided evidence for significant increase of skeletal lead mobilization during pregnancy. However, the amount of lead contributed to plasma (i.e., the partitioning of lead in the blood pool) was indirectly measured using unique samples (*29*). In addition, only a small number of subjects were willing to undergo tibial XRF measurements because of concern over past radiation exposure (*30*).

Multiple linear regression models may not permit a full appreciation of the influence of maternal bone lead levels on the fetal cord blood lead levels through maternal blood or plasma lead levels, because they involve the use of one dependent variable and multiple independent variables in a single equation. Because maternal blood lead is an intermediate marker for the source of lead exposure, the model may be overcontrolled by the inclusion of all independent variables with equal roles in the matrix (*20*). For example, bone lead must be released into maternal plasma to influence the fetal cord blood lead levels; however, in a multiple regression model, the influence of bone lead levels on the fetal cord blood lead levels cannot be revealed after controlling for maternal blood lead. In the SEM, plasma lead can be inserted as a latent variable in a set of relative equations, and covariance matrix and maximal likelihood estimation can be used to calculate coefficients that indicate the association between plasma lead and the other observed variables.

The results of our study suggest that plasma lead level is an important biomarker for endogenous and exogenous lead exposure in pregnant women and their fetuses. Insertion of plasma lead level as a latent variable in the SEM allowed us to examine simultaneously the association between maternal bone lead and external lead exposures on fetal cord blood lead without bias. However, this method cannot offer an actual plasma lead value. Although currently difficult, plasma lead measurement may be necessary to elucidate particular lead toxicity issues, such

as the mobilization of lead from bone and fetal lead exposure during pregnancy.

The results of our study also suggest that if one wants to predict fetal lead exposure (as signified by cord blood lead levels) before pregnancy occurs, measuring maternal bone lead levels as well as maternal blood lead levels and current environmental sources of lead exposure may provide a powerful set of tools. Our analysis using cross-sectional epidemiologic data certainly has limitations from a biokinetics point of view because we do not have repeated measurements with which to measure dynamic relationships and are unable to glean from our data any immediate biokinetic relationships, such as those between fetal RBC lead and fetal plasma lead. Moreover, a simple cord blood lead level alone is likely to have limited ability to represent fetal lead exposure throughout pregnancy. Nevertheless, from a clinical and epidemiologic point of view, maternal bone and blood lead levels and environmental data remain the only tools available with which to assess individual risks before or early in pregnancy. Such risk assessments, in turn, are important, given the attention that is now starting to be directed toward developing strategies for preventing maternal bone lead mobilization during pregnancy using approaches such as nutritional supplementation (*31*).

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