Study of the Effect of Lactational Bone Loss on Blood Lead Concentrations in Humans

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Lactation and other clinical states of high bone turnover have been suggested to release lead (Pb) stored in bone into blood and tissues. Previous observations on the influences of lactation have been anecdotal, or at high blood Pb concentrations with varying past exposures, or complicated by postpartum fluid changes. A prospective observational study was performed to investigate possible changes in blood lead concentrations at multiple intervals during lactation for 6 months postpartum and to relate changes in blood lead concentrations to changes in bone density and other variables. Volunteer pregnant subjects (n = 58) were enrolled from a midwifery service at an academic public health hospital. Subjects were mostly Hispanic, recently immigrated, of low economic status, not receiving supplemental calcium, and had low blood Pb concentrations (2.35 ± 2.05 μg/dl at enrollment). Bone density losses over 6 months for the group averaged -2.46 ± 6.33% at the vertebral spine and -0.67 ± 5.21% at the femoral neck. In predicting final bone density, apart from initial bone density only the total number of breast-feedings was a significant independent variable of the variables tested, accounting for an additional 12% of the variability. No changes in blood Pb concentrations were seen over the interval beyond 2 weeks postpartum (minimum detectable change was 0.4 μg/dl). There was no relation between the changes in bone density and changes in blood Pb or the integrated blood Pb over the 2-week to 6-month period. Normal (nonlactating) bone resorption rates contribute a large fraction of the Pb in blood during low-exposure circumstances. However, during lactation the increase in bone resorptive processes is probably relatively small with a larger decrease in deposition accounting for net bone loss, as suggested by other investigations. Thus, concomitant release of Pb from bones of lactating subjects with low blood lead concentrations on this background of high normal resorption was not large enough for detection. Key words: blood lead, bone density, lactation, Pb. Environ Health Perspect 107:187–194 (1999). [Online 26 January 1999] http://ehpnet1.nih.gov/docs/1999/107p187-194osterlob/abstract.html

Greater than 90% of the metal lead (Pb) in the adult human body is stored in the bone (1). The equilibria between bone and other tissues greatly favors bone as a sink (2,3), but even the small rate of Pb released back to tissues from the large bone compartment may contribute significantly to the tissue or blood concentrations (bone concentrations are approximately 100–200-fold greater than blood). Using the natural isotopic signatures of endogenous Pb, we have previously shown that for individuals with normal background exposure to Pb, approximately 60% of Pb in the blood is derived from earlier deposited Pb in bone stores (4). Pathologic and physiologic states such as hyperparathyroidism, pregnancy, lactation, and osteoporosis have been suggested to mobilize Pb from bone stores (5–8). Isolated observations in several individuals with high past Pb exposure have shown increases in blood Pb concentration thought to be due to clinical states of increased bone turnover (9–12). Longitudinal studies are being performed to examine the influences of osteoporosis and pregnancy more closely.

Decreases in bone density of 0–5% during lactation have been clearly documented by several investigations using x-ray densitometry, particularly when lactation exceeds 5–6 months (13). With the mobilization of calcium during lactation, Pb mobilization might also be expected. In a recent cross-sectional study of bone Pb concentrations in a normally exposed population measured by K-shell x-ray fluorescence, age and sex were the most important predictors in modeling the accumulation of Pb in bone. However, lactation history was also a weak inverse predictor in females of the sampled population (14). Potential increases in blood Pb concentrations during lactation might roughly be predicted from measurements of bone Pb concentrations, the partitioning of released Pb between the blood and various tissue pools, and the amount of bone loss measured during lactation. Limiting knowledge includes the precise dynamic partitioning of lead in blood and tissues, poor information on whether Pb is released from bone in a manner similar to calcium (may be less soluble to osteoclastic influences), whether Pb is redeposited back into bone from the fractions previously released, and other variables.

Only one study has attempted to assess changes in blood Pb concentrations during lactation in humans (15). In this study only a small portion of the subjects were actually breast-feeding and for only short durations. In addition, changes in bone density were not measured and initial baseline blood Pb concentrations were confounded by acute peripartum changes in fluid volumes. To address the concern that lactation may mobilize lead, thereby possibly increasing harmful tissue levels, we have undertaken a longitudinal study of a relatively uniform cohort of lactating women who were not receiving calcium supplementation, assessed with measurements of breast-feeding frequency, serial blood Pb concentrations, and validation of bone loss by dual-energy bone densitometry.

Materials and Methods

Setting and design. This study took place at San Francisco General Hospital through the Nurse Midwifery Service (NMS) from June 1994 to May 1996. Study protocol and consent procedures were approved by the University of California Committee on Human Research. The design was that of a prospective cohort with measurements of breast-feeding frequency and blood Pb concentrations at 2–3 weeks prior to delivery, at birth, and at 2 weeks, 6 weeks, 3 months, and 6 months after delivery. Bone densitometry was measured at delivery and at 6 months. Bone marker measurements were made at 2 weeks and 6 months after delivery. The major outcome was the relationship between the change in blood Pb concentrations over the 2-week (or 6-week) to 6-month postdelivery interval and the change in bone densities at vertebral and femoral neck sites. Uncomplicated pregnancies following prenatal visits in the NMS were the source of the subject population. This service performs approximately 600 assisted hospital deliveries/year and

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serves a surrounding community of low income, primarily Hispanic people, most of whom are uninsured and qualify for the Well Infant Child care program (annual household income <$17,500/year).

Enrollment and protocol. Pregnant patients receiving prenatal care were given preliminary information about the study in the form of a study announcement distributed by the Certified Nurse Midwives (CNM) or the research assistant during prenatal visits. A few subjects were referred from the Obstetrical Service, given the preliminary announcement, and thereafter treated as NMS patients. In addition, the study announcement was posted on clinic doors and in waiting areas. Those contacting the research assistant at the time of the initial contact or via phone were scheduled at 32–38 weeks of gestation for an explanatory meeting and given the informed consent form to review. Those consenting immediately and those consenting after rescheduling were interviewed for medical, dietary, social, and breast-feeding histories. Blood samples were also obtained at this time for blood Pb, hemoglobin, serum albumin, and iron concentrations. Clinic charts on these subjects were reviewed within the week after the interview for specified medical data and compared for consistency with medical interview information. All interview responses and chart information were entered directly into a laptop computer after the research assistant verbally read each prompted question. After this interview and as an overall educational effort by the NMS to encourage breast-feeding, all clinic clientele were given brochures and viewed a videotape on proper breast-feeding. All information, consent forms, and interview questions were developed and provided in Spanish or English.

Inclusion/exclusion criteria applied during the initial interview process were as follows: all subjects must be pregnant, 18–40 years of age, and in generally good health. Subjects with a history or treatment of any serious acute or chronic disease, including but not limited to congestive heart failure, insulin-dependent diabetes, renal insufficiency, metabolic bone disease, or endocrine problems, were not eligible. Pregnant women with underlying diseases or anticipated complications of pregnancy were not admitted to the NMS. Testing for conditions prevalent in this hospital population, such as hepatitis B, human immunodeficiency virus positivity, or intravenous drug abuse, were not performed, although subjects were excluded if they gave a positive history. All potential continuing subjects must have had at least one prior prenatal visit.

Prenatal charts were flagged with instructions to obtain blood tests and alert the research assistant when the subject was admitted for delivery. Within 1–2 days of delivery, further specified data regarding delivery complications, maternal and infant weights, and a hospital nutritionist evaluation were obtained from the peripartum chart. A portion of the earlier interview on breast-feeding variables was repeated at this time and bone densities at femoral neck and vertebral sites were performed. Interviews to obtain continuing information on breast-feeding variables and blood sampling were repeated again at 2 weeks, 6 weeks, 3 months, and 6 months. In addition, the 6-month interview included repeated dietary and medical evaluations, a pregnancy test, and the second bone density determination.

All subjects were scheduled for interviews at the beginning of the study with follow-up tracking and reminder phone calls made prior to scheduled visits. Nearly all of the 1,007 patients seen by the NMS during the enrollment period had viewed the posted announcement or were approached by the CNM or research assistant. One hundred forty-five potentially interested subjects were read the consent form and 120 of these enrolled. Those not enrolling cited lack of time, fear of blood drawing, and partner opposition as reasons. Of the 120 enrollees, 58 completed the entire study, 19 discontinued the study before the first or second interview, and 43 finished the study but missed multiple or critical scheduled dates (incompletes). Of the total 62 not completing or discontinuing the study, reasons cited for noncompletion were enrollment relocation (n = 6), partner opposition (n = 1), fear of blood drawing (n = 6), time conflicts (n = 3), infant death (n = 1), no reason given (n = 5), and the remainder were due to missed appointments.

Questionnaire and chart information. A goal in this study was to obtain a more precise measure of breast-feeding than in a model lactation study (1.3), where only the time to discontinuation of breast-feeding was recorded. The reason for this was to demonstrate the validity of this model in our application, i.e., that bone loss had actually occurred and was related to breast-feeding. Measures of breast-feeding frequency (total number of breast-feedings) were computed from the number of breast-feedings recalled for each interval during the day, at night, and the estimated ounces and number of breast pumpings performed. Time to the discontinuation of breast-feeding and the number of bottle feedings were also recorded. Other variables obtained from the questionnaire or medical charts were gathered to address the medical exclusionary criteria and to obtain other independent variables that might impact either the breast-feeding–bone loss relationship or the major outcome relationship of whether bone loss is associated with changes in blood Pb (Table 1). In addition, four composite variables were computed: socioeconomic rating, health rating, calcium intake rating, and dietary quality rating. Each composite variable was an attempt to rank order or stratify the subjects within the narrow variation of this cohort. Socioeconomic rating was computed by averaging the five z scores of the following variables: 1) the number of adults working divided by the number of adults and children in the home; 2) the number of rooms divided by the number of adults and children in the home; 3) the monthly rent divided by the number of adults and children in the home; 4) the number of years of education of the subjects; and 5) the number of years living in the United States [as partially adapted from Centerwall (16) (17). The health rating was calculated by averaging the two z scores of the following variables: the number of lifetime illnesses (more serious than colds, flu, and common childhood illnesses) added to the number of minor illnesses during the current pregnancy; and the number of times that the serum albumin and iron concentrations were outside the relevant reference range during the 6-week, 3-month, and 6-month visits. Calcium intake and dietary quality ratings were not based on explicit food frequency inventories. Dietary quality ratings resulted from scoring interview questions covering the number of meals and snacks, foods most liked, and milk and fast-food consumption. Calcium ratings were weighted toward dairy product consumption because milk ingestion predicts 80% of the variability of calcium intake and ratings included amounts of milk ingested and foods most consumed (18–20).

Bone density measurements. The dual-energy x-ray absorption measurements made within 1 day of delivery and at 6 months postdelivery were performed on a Lunar DPX dual-energy x-ray densitometer (Lunar Corp., Madison, WI) in accordance with the manufacturer’s instructions and after certification training of our research technician (21). The instrument utilizes an x-ray tube source with a cerium filter producing two x-ray energies of 38 and 70 keV, and a sodium–iodide crystal photomultiplier detector. The x-ray tube was operated at 76 kV and 750 μA and collimated to 1.68 mm. The exposure per scan was 1.2 mR (22). Bone density (in grams per square centimeter) was measured at the right femoral neck and the vertebral lumbar spine (average of L2–L4). Subject positioning was standardized. For
the anterior–posterior scan of the spine, the subject was placed in a supine position with the legs flexed at the knees; for the anterior–posterior scan of the hip, the subject was supine with the hip internally rotated and braced at the foot. Calibration checks and quality control phantom were performed with each day of use. Short- and long-term precisions were 0.32–1.16% coefficient of variation (CV) and were within reported values (23). A comparison of a set of phantom readings separated by 15 months showed a 0.33% bias.

**Blood Pb measurements.** Blood drawn into separate Becton–Dickinson blue top trace metals vacutainer tubes (Becton–Dickinson, Franklin Lakes, NJ) containing EDTA was refrigerated at 2–8°C. Analysis was performed by graphite furnace atomic absorption spectrophotometry with Zeeman background correction on a Perkin–Elmer Model 3030Z with an HGA 600 furnace (Perkin–Elmer, Norwalk, CT) (24). Sample transfer tubes and reagent glass or plasticware were acid-washed prior to use. Preparatory work was performed in a high-efficiency particulate air (HEPA)-filtered class 10,000 clean space. All six samples on the same person were performed within the same run. Samples were prepared in duplicate and each duplicate was analyzed twice. Quadruplicate results were averaged. Intra-assay precision was 6% (CV) at 2.0 μg/dl. Accuracy was maintained by participation in the College of American Pathologists/American Association of Clinical Chemists Blood Lead Survey approved by the Occupational Safety and Health Administration and by the use of assayed proficiency test samples as calibrators.

**Other blood and serum measurements.** Hemoglobin, albumin, and iron measurements were performed in the hospital clinical laboratory by standard methods. Osteocalcin (NovoCalcins, Metra Biosystems Inc., Mountain View, CA) and bone-specific alkaline phosphatase (ALKPHASE-B, Metra Biosystems Inc.) measurements in serum at 2 weeks and 6 months were determined at the University of California San Francisco Calcitrophic Hormone Reference Laboratory. After phlebotomy, specimen tubes were placed immediately in an insulated cool pouch and were centrifuged and separated into serum within 1 hr. These were then stored at -20°C until analysis. All samples were run as paired specimens and processed in duplicate. Both assays are enzyme-linked immunosorbent assays having intra-assay CVs of 4.6 and 7.6%, respectively.

**Data processing and statistical methods.** All data were entered into predefined template forms on a laptop computer (interview, chart review histories, routine laboratory data, and bone density measurements) and were managed by Epi Info software Version 6 (Centers for Disease Control and Prevention, Atlanta, GA). Initial errors and completeness were checked with this program. Data were transferred to Statistical Analysis Systems software (SAS Institute, Cary, NC) for subsequent analysis. Error checking was then performed manually and random spot checking was performed against original sources when available.

All variable distributions were reviewed for normality and only blood Pb concentrations required logarithmic transformation. Other descriptive statistics were computed as means and standard deviations. Comparisons between completed and completed study subjects were performed on prepartum data by t-test or chi-square test. The determination of randomness of completion was performed by comparing means of the sequentially assigned subject number. Validity of the study design (lactation-related bone density changes) was confirmed by comparing the mean change in bone densities for subjects breast-feeding at 6 months to those quitting before 6 months, as in Sowers et al. (13). In addition, the validity of the design was examined using multiple regression analysis of the change in bone densities as influenced by breast-feeding frequency and other independent variables.

Percentage changes in blood Pb concentrations over postpartum time intervals were examined by multiple regression analysis with respect to percentage changes in bone densities and other possible independent variables. Also, integrated blood Pb concentrations were computed over the 2-week to 6-month period and used as the outcome variable in place of the percentage change in blood Pb and then examined similarly. As little as 15% (R² of >0.15) of the variability in the relationship between the percentage changes in bone density and percentage changes in blood Pb could be detected by this analysis, when n = 58, α = 0.05, and β = 0.80. Given the sample size, mean, and standard deviations of the blood

| Variable (units) | Study group (n = 58) | Incompletes (n = 43) |
|------------------|---------------------|---------------------|
| Age at delivery (years) | 25.8 ± 6.6 | 26.7 ± 5.7 |
| Ethnicity (% Hispanic) | 81.0 | 76.7 |
| Time living in the United States (years) | 8.4 ± 10.0 | 8.3 ± 9.1 |
| Years of education (years) | 9.8 ± 3.1 | 10.2 ± 3.3 |
| Proportion in WIC (%) | 87.9 | 88.6 |
| Monthly rent per family (dollars) | 607 ± 307 | 585 ± 305 |
| Number of rooms in home (n) | 3.6 ± 1.5 | 3.7 ± 2.8 |
| Number of adults living at home (n) | 3.0 ± 1.3 | 3.7 ± 2.1 |
| Number of adults employed (n) | 1.7 ± 1.1 | 1.9 ± 1.3 |
| Within-cohort socioeconomic rating (Z-score) | 0.07 ± 0.72 | -0.03 ± 0.55 |
| Having ever smoked (%) | 19.0 | 45.2* |
| Having ever drank alcohol (%) | 49.0 | 59.0 |
| Having ever used illicit drugs (%) | 6.9 | 16.3 |
| Health rating (z score) | 0.00 ± 1.07 | 0.04 ± 0.96 |
| Gravdity (n) | 2.6 ± 1.6 | 2.4 ± 1.7 |
| Time since last delivery (days) | 1333 ± 1090 | 1708 ± 1385 |
| Number of living children (n) | 1.0 ± 1.3 | 0.7 ± 1.2 |
| Number of prenatal visits (n) | 11.3 ± 3.6 | 12.0± 2.3 |
| Prenatal weight (lb) | 164 ± 32 | 170 ± 34 |
| Proportion having spontaneous vaginal delivery (%) | 79.3 | 76.7 |
| Neonatal birth weight (g) | 3515 ± 450 | 3426± 474 |
| Time in hospital after delivery (days) | 1.5 ± 0.8 | 1.9 ± 1.3 |
| Prenatal serum albumin (g/dl) | 3.2 ± 0.7 | 3.2 ± 0.2 |
| Prenatal serum iron (μg/dl) | 94 ± 59 | 89 ± 52 |
| Prenatal hemoglobin (g/dl) | 14.0 ± 5.9 | 13.9± 5.7 |
| Using prenatal iron supplements (%) | 15.5 | 18.6 |
| Using calcium supplements during lactation (%) | 5.2 | 9.3 |
| Calcium intake rating (score units) | 3.6 ± 1.2 | 3.8 ± 1.6 |
| Dietary quality rating (score units) | 2.6 ± 0.7 | 2.9 ± 0.7 |
| Bone density at femoral neck (g/cm²) | 0.97 ± 0.13 | 0.97± 0.11 |
| Bone density at vertebral spine (g/cm²) | 1.11 ± 0.12 | 1.17± 0.16 |

Abbreviations: SD, standard deviation; WIC, Well Infant Child Program.

*P<0.05, chi-square test.

aMean days since last delivery for those having previous pregnancy.

bN=43, not all incomplete subjects reached this stage.

*To convert serum albumin from g/dl to g/l multiply by 10; to convert serum iron from μg/dl to μmol/l multiply by 0.179; to convert blood hemoglobin from g/dl to g/l multiply by 10.
Pb concentrations, the minimum mean detectable change in blood Pb concentrations between any two time points was 0.02 μmol/l (0.4 μg/dl).

**Results**

The study subjects were primarily Hispanic immigrants, noncollege-educated, healthy, and of poor economic status. Descriptive statistics of the incomplete and completed study subjects are given in Table 1 as obtained by interview questionnaire and chart review. There were no differences between the study group and the incomplete group except for recall of having ever smoked. No subject reported smoking during pregnancy or lactation. Occurrence of incompletion was random. The childbirth and peripartum variables listed in Table 1 are typical of the population seen at the NMS.

Table 2 shows the important outcome variables during lactation. The average subject was estimated to have 1,002 breast-feeding episodes. As expected, the percentage of subjects breast-feeding and the number of breast-feedings per day steadily diminished as the postpartum periods progressed. At the end of the study, 63.8% were still breast-feeding. Table 2 shows the mean blood Pb concentrations of study subjects at each interview time point. Blood Pb concentrations were similar to local population values. For the study subjects, the blood Pb concentrations demonstrated a significant 15% decrease from the prepartum appointment date to the delivery day, a 13% increase from the prepartum appointment to the 2-week postpartum date, and a further 8.9% increase from the 2-week date to 6 weeks postpartum (Table 2). Only an additional 0.3% increase (not significant) was seen from 6 weeks to 3 months. There were no significant changes in paired blood Pb concentrations over the intervals from 2 or 6 weeks to 6 months postpartum for the cohort. Figure 1 shows the temporal correspondence of the changes in blood Pb and the changes in serum albumin.

**Study validation.** Overall, the entire group showed a significant decrease in the bone density in the vertebral spine (2.46 ± 6.33%), but not in the femoral neck. Osteocalcin and bone-specific alkaline phosphatase concentrations tended to be modestly higher at the 6-month versus the 2-week time point (Table 2). Changes in the bone density of the vertebral spine tended to be greater in those subjects still breast-feeding at 6 months (3.04 ± 6.93%, n = 36) than in those breast-feeding less than 6 months (n = 22), as can be seen in Table 3. Femoral neck bone density changes also tended to be greater in the subjects breast-feeding through 6 months than in the subjects with lesser amounts of breast-feeding, though also not statistically significant.

The relationship between bone densities at delivery and at 6 months was expected to be strong (simple \( R^2 = 0.6550, p = 0.0001 \), with breast-feeding frequency accounting for an extra 12% of the variability (multiple \( R^2 = 0.7799, p = 0.0001 \)) and the other variables (age, weight change, gravidity, time since last delivery, initial serum iron concentration, calcium intake rating, dietary quality rating, initial hemoglobin concentration, and health rating) not contributing significantly. Thus, the determinants of the final bone density in the vertebral spine were the initial bone density and breast-feeding frequency. The analogous relationships for the bone densities of the femoral neck with breast-feeding frequency were \( R^2 = 0.8673, p = 0.0001 \), and \( R^2 = 0.9094, p = 0.0001 \), respectively. Although breast-feeding intensity was a significant independent variable, its impact in the femoral site was less (accounting for only 4% additional variability).
Outcome relationships. The percentage change in blood Pb concentrations across the meaningful period of lactation (i.e., from either 2 weeks to 6 months or 6 weeks to 6 months) was not related to percentage changes in bone densities (either vertebral spine or femoral neck) when tested by simple regression or after multiple regression including the following additional variables: age, percentage weight change, gravidity, average serum iron concentration, socioeconomic rating, calcium intake rating, calorie intake rating, years in the United States, and percentage change in serum albumin concentration for the same period (Table 4). Using the blood Pb concentration at either 2 or 6 weeks to predict the concentrations at 6 months showed an expected significant regression (e.g., blood Pb from 6 weeks and 6 months, $r^2 = 0.6495$ as simple regression), but also without impact from other listed variables including the change in bone densities when examined by multiple regression ($R^2 = 0.6718$). Multiple regression of the integrated blood Pb from 2 weeks to 6 months with the same independent variables showed that only weight change was significant (Table 4).

Discussion
This study demonstrates no observable change in blood Pb concentrations from 2 or 6 weeks to 6 months postpartum and no relationship between the change in bone densities and the change in blood Pb concentrations during lactation. Thus, it is unlikely that measurable effects would result from Pb released from bone due to lactation at these low (well within population norms) blood Pb concentrations. The study also confirms that changes in bone density do occur during lactation and are related to breast-feeding frequency.

This study was modeled in part after the investigation of Sowers et al. (13), in which changes in bone density of approximately 5% were seen in lactation periods of up to 5–6 months. Our study showed slightly less bone density losses over 6 months and showed the same ordered magnitudes at vertebral spine and femoral neck sites. We attempted to improve upon that study by encouraging breast-feeding in all subjects, by using a more dynamic account of breast-feeding, and by focusing on a relatively homogeneous population (similar socioeconomic, ethnic, educational, and health backgrounds) cared for by a single service. The results showed that both the continuous measure of breast-feeding frequency and the dichotomous measure of greater or less than 6 months of lactation were predictors of bone losses. We had considered that this population of subjects might also show greater bone losses than in the model study and might have higher blood Pb concentrations than the general population because subjects were economically poor and did not take calcium supplements. However, neither enhancement was observed. Subsequently, kinetic studies by Specker et al. (25) and a prospective study by Kalkwarf et al. (26) showed that the mobilization of bone calcium during lactation is little affected by supplemental calcium intakes.

Younger age (<18 years) (27), greater gravidity or parity (28,29), lower calcium intake (25,27,28), and shorter interpregnancy intervals (30,31) have inconsistently been associated with bone density losses. None were significant factors in our regression analysis of changes in bone density as a function of breast-feeding intensity and other variables. This may be due to the small sample size of this study, a lack of any relationship, and possibly in part due to the greater precision of breast-feeding estimates.
Adequate calcium intake will reduce dietary Pb absorption as compared to deficient intakes in animal studies (33,34). Thus, a measure of calcium intake may independently predict blood Pb accumulation from dietary sources during the lactational period. On the other hand, variation in calcium intake is not thought to be a predictor of Pb mobilization from bone per se because calcium intake during lactation had neither altered calcium loss from bone in humans (25,28) nor increased the mobilization of Pb during lactation in animal studies (8). Most subjects had a reasonable dietary intake of dairy products, though only a few were ingesting additional supplemental calcium tablets. However, dietary estimates of calcium intake are notorious for their imprecision and are weighted heavily toward historical recall of dairy products (18). Given this information and the limited extent to which calcium intake was queried, an effect of this variable was also unlikely. Average serum iron concentrations were used as a measure of adequate iron intake, which also can reduce dietary absorption of Pb (35).

Changes in serum albumin over the same periods (as for changes in blood Pb) were used as a surrogate independent variable to account for both plasma volume and hematocrit changes. Tissue mass and body water increase in rough proportion during pregnancy, but there is a slight excess of increased body water. Along with an iron-limited decrease in erythropoiesis during pregnancy, this results in a 15% decrease in hematocrit by the 34th week of pregnancy, which is maintained until delivery. Much of this dilution and lowered hematocrit is substantially recovered by 4 weeks postpartum (36-38). Use of albumin as a surrogate was chosen in this study because hematocrit or hemoglobin were collected only at initial time points and because albumin varies similarly. The need to account for plasma volume changes is clear because 95% of the Pb resides within the red blood cell. This is apparent in Figure 1, where the changes in albumin concentration were trended in the same direction as the changes in blood Pb for each period where the volume changes were most dramatic (enrollment to delivery, and delivery to 2 weeks postdelivery).

In post hoc analysis, these peripartum intervals known to be affected by changes in fluid volume were also examined. During the period between 2 and 6 weeks, the percentage change in blood Pb was significantly regressed with the percentage changes in the vertebral bone density ($p = 0.0103$ for the model, $p = 0.0466$ for vertebral bone), but not changes in femoral bone density. However, most of the variability in this multiple regression was accounted for by the percentage change in albumin ($p = 0.0096$) over the same interval. This regression has limited biologic or logical meaning because there is no temporal relation between the prediction of short-term changes in blood Pb (over 4 weeks) by long-term changes in bone density (6 months). One possible interpretation may be that early lactational stimuli are pronounced and measurable mobilization had in fact occurred. Yet measurable bone losses by bone densitometry are not detectable for such short durations (13). Because of the strong impact of the changing plasma volume surrogate, this observation may have a conservative interpretation.

The data collection time points in this study were chosen for several reasons. The 6-month measurements were selected as the time previously demonstrated where bone losses were maximal as measured by dual-energy x-ray densitometry (13). The initial bone density measurements were made within 1-day postpartum for most subjects because individuals were already in the hospital near the instrument. Prepartum bone density determinations were not performed to avoid concern over fetal radiation exposure, which would have been extremely low, although the perception of the subjects would probably have affected recruitment. Osteocalcin and bone-specific alkaline phosphatase at 2 weeks and 6 months were measured in an attempt to add further confirmation regarding increasing bone activity (39). Limitation in expenses and utility of these markers led to the decision to measure these only toward the beginning and end of the study. Thus, neither peak activity nor maximum change in activity due to lactation were necessarily assessed.

Sampling time points for blood Pb concentrations were spaced to find a baseline sampling time less affected by the aforementioned acute postpartum physiologic changes in body mass and blood volume. In the only prior study of blood lead changes during the lactational period (15), conclusions about changes during lactation were limited because of the small amount of breast-feeding that occurred (25% of the subjects breast-fed and only 8% fed longer than 4 months), the lack of confirmatory bone mass measurements, and the poor choice of blood Pb sampling time at the delivery date. Peripartum blood Pb concentrations are hemodiluted due to the aforementioned volume changes of pregnancy and decreased erythropoiesis. Later postpartum concentrations are likely to show lessening degrees of hemodilution. In addition, in some cases blood Pb concentrations are likely to be diluted further at delivery by
intravenous fluid replacement that is often provided. Our data show that blood Pb concentrations had decreased by the first day after delivery as compared to prepartum values by 15.0%. By 2 weeks postpartum, blood Pb concentrations have regained and surpassed the pre- and peripartum hemodiluted concentrations. An appropriate sampling time to observe changes in blood Pb due to lactational influences would not be the delivery date used in the earlier study, but after some equilibration such as the 2- or 6-week postpartum sampling times available in this study. Also, all these same intervals were used as convenient interview times for questions directed at assessing breast-feeding intensity.

Previous information in humans on the possible mobilization of Pb from bone due to various conditions is mostly anecdotal or cross-sectional, but is occasionally convincing in case reports of higher body Pb burdens during pathophysiologic states that cause increased bone turnover (11,12). That blood Pb may be influenced by hormonal factors affecting bone turnover has been observed by measuring a decrease in blood Pb concentrations in hyperparathyroid subjects after becoming euparathyroid (7). Also, cross-sectional studies and one prospective study on Pb mobilization during pregnancy have shown slight increases in blood Pb during the third trimester (6,40), although levels tended not to exceed first-trimester blood Pb concentrations. Recently, Gulson et al. (41) showed that isotopically distinct bone sources of Pb contributed to blood Pb in immigrants who acquired the local prevalent blood isotopic composition during their pregnancies, although actual blood Pb concentrations only increased slightly and variably.

One case report of elevated blood Pb concentrations during breast-feeding in a woman with past occupational exposure to Pb is documented (42). Baseline blood Pb concentrations in this case were approximately 15 times greater than in our cohort, so these greater amounts of Pb recently deposited in bone may have allowed easier detection. However, blood Pb concentrations only slightly exceeded prepartum concentrations and peripartum hemodilution may have been a factor in properly interpreting that observation. Animal studies have demonstrated that after long-term oral intake, Pb can be mobilized from bone during lactation (8,43) with three significant differences from the human condition: doses and tissue concentrations were manyfold higher; rodents tend to mobilize larger amounts of bone than humans; and discontinuation of high Pb intakes just prior to lactation or continuation of exposure during lactation is likely to result in mobilization of recently deposited Pb, whereas if the Pb were deposited in the distant past or nonhomogeneously, mobilization of Pb may be less likely.

Using blood Pb as a measure of Pb released from bone may have limitations in our study and in all such investigations. Because of the low concentrations currently prevalent in U.S. populations of child-bearing age, measurement of real change in blood Pb is difficult because of analytical and biologic variability. Although strategies such as quadruplicate blood Pb analysis, same-run analysis of all samples from a subject, and the use of a homogeneous population from one section of San Francisco were aimed at reducing analytical and biologic variability, a minimum change of only 0.4 μg/dl would have been detectable in this cohort size. Yet, while this represents a relatively large change as compared to starting concentrations, it does demonstrate that serious elevations in blood Pb are not occurring in lactating populations with the currently low blood Pb concentrations. Another limitation to using blood Pb as an end point is the dilution effect of tissues (other than blood) on Pb released from bone. Tissue volumes and tissue Pb concentrations are larger than the blood Pb pool and may take up much of the released Pb. At higher bone concentrations of Pb or a with more recent exposure on a background of lowered contemporary blood Pb, such mobilization may be detectable.

Whether Pb may be released in some proportion to calcium loss from bone is unclear. Although Pb follows calcium into bone, Pb salt dissolution constants are much poorer than calcium salts, so less may be released under osteoclastic influences (acid dissolution). This may be the inherent reason underlying the observations that Pb accumulates in bone relative to calcium throughout life. On the other hand, a cross-sectional autopsy study of bone Pb analysis by age showed that after the sixth decade of life Pb no longer accumulated, but that the amount of Pb ratioed to calcium (mineral or ash) tended to decrease in trabecular bone, suggesting disproportionately greater loss of Pb than calcium during those years of aging and osteoporosis (44). However, possible secular periods of past exposure make these data difficult to interpret with respect to kinetic processes, although they are consistent with current thought by many investigators regarding Pb mobilization. Given that bone contributes a sizeable proportion of the Pb in the blood compartment during low environmental exposure (4,41), it may be that the relatively slight increases in resorption seen in various pathophysiologic states may not be that much greater than the already high baseline contributions. This would suggest that the mechanism for calcium availability during lactation is reduced deposition, which is consistent with both the noted lack of effect of calcium supplementation on bone density during lactation (25,26) and with the suppression of calcium urinary losses during lactation (28).

No actual assessment of changing external environmental Pb exposures was feasible in this study. Therefore, low levels of exposure to Pb for any subject may not have been at a steady state during the course of the study. For instance, a few subjects changed their living quarters during the study, but did so within the same section of the city. Still, different background Pb exposures may have resulted and may have varied enough to increase or decrease baseline blood Pb concentrations. Whereas individual circumstances would average, seasonal or broad environmental trends might bias the measured change in blood Pb. Other limitations include the previously stated power limitations, the prevalence of low blood Pb concentrations, the limited impact of the other independent variables examined, and the limited ability to detect nonlinear relationships. Also, the generalizability of this study is confined, in that other populations of different ethnicity, circumstance, or bone Pb stores (large past exposures) may behave differently. This study only demonstrates that serious elevations in blood Pb concentrations do not occur as a result of lactation.

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