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Original Contribution

Influence of Maternal Bone Lead Burden and Calcium Intake on Levels of Lead in Breast Milk over the Course of Lactation

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The authors studied 367 women who were breastfeeding their infants in Mexico City, Mexico, between 1994 and 1995 to evaluate the effect of cumulative lead exposure, breastfeeding practices, and calcium intake on breast milk lead levels over the course of lactation. Maternal blood and breast milk lead levels were measured at 1, 4, and 7 months postpartum. Bone lead measurements were obtained at 1 month postpartum. At 1, 4, and 7 months postpartum, respectively, the mean breast milk lead levels were 1.4 (standard deviation (SD), 1.1), 1.2 (SD, 1.0), and 0.9 (SD, 0.8) μg/liter and showed a significant decreasing trend over the course of lactation ($p < 0.00001$). The relations of bone lead and blood lead to breast milk lead were modified by breastfeeding practice, with the highest breast milk lead levels among women with a high level of patella lead who were exclusively breastfeeding. Dietary calcium supplementation increased the rate of decline in breast milk lead by 5–10%, in comparison with a placebo, over the course of lactation, suggesting that calcium supplementation may constitute an important intervention strategy, albeit with a modest effect, for reducing lead in breast milk and thus the potential for exposure by infants.

bone and bones; breast feeding; calcium; lactation; lead; longitudinal studies; milk, human

Abbreviations: K-XRF, K x-ray fluorescence; SD, standard deviation.

Maternal bone lead burden and breastfeeding practices have been shown to be important predictors of maternal blood lead levels over the course of lactation (1). Thus, it would be expected that, in addition to any current intake, the lead accumulated in bone from past exposures and released into blood is subsequently excreted into breast milk. Since over 90 percent of lead in the adult human body is stored in bone (2, 3), the possibility exists for considerable redistribution of cumulative lead stores during periods of heightened bone turnover (e.g., pregnancy and lactation). In fact, it has been demonstrated that the major sources of lead in breast milk are from maternal bone and diet (4) and that the mobilization of lead from bone continues in the postpartum period for up to 6 months (5). However, there are limited data available to quantify the hazard this represents for the breastfeeding infant over the course of lactation or to understand opportunities for preventing these potential exposures.

Previously we have reported that, even among a population of women with a relatively high lifetime exposure to lead, levels of lead in breast milk were low, influenced by both current lead exposure and redistribution of bone lead accumulated from past environmental exposures (6). Although these levels were low, they clearly had a strong

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influence on infants' blood lead levels over and above the influence of mothers' blood lead (7).

In a randomized, controlled trial of calcium supplementation during lactation, we have also shown that dietary calcium supplements were associated with 15–20 percent lower maternal blood lead levels over the course of lactation (8). These results suggest that potentially modifiable nutritional factors may be important targets for intervention to prevent mobilization of lead in nursing women and, thus, in preventing undue exposures to breastfeeding infants.

This study provides the opportunity to assess the contribution of maternal bone lead to lead in breast milk over time by use of multiple biomarkers of exposure and to determine whether the degree of this influence changes over the course of lactation. We conducted a longitudinal analysis among 367 lactating women in Mexico City, Mexico, at 1, 4, and 7 months postpartum to evaluate the relations among maternal blood, bone, and breast milk lead levels. In addition, we examine dietary calcium intake and breastfeeding practices as potential modifiers of the associations over time.

MATERIALS AND METHODS

Subjects were a subset of the participants in a study of calcium supplementation during lactation who breastfed their infants and provided breast milk samples for lead analysis. All participants received a detailed explanation of the study and counseling on reduction of lead exposure, and informed consent for participation was received. The research protocol was approved by the human subjects committees of the National Institute of Public Health of Mexico, Harvard School of Public Health, and participating hospitals.

Data collection methods have been described in detail elsewhere (1, 8). Subjects ($n = 629$) were enrolled after delivery between January 1994 and June 1995 in Mexico City and completed a baseline evaluation, including a questionnaire that assessed known risk factors for environmental lead exposure and breastfeeding practices. Maternal dietary intake was assessed by use of a semiquantitative, food frequency questionnaire designed to estimate usual dietary intake over an extended period of time prior to completion of the questionnaire (9).

At 1 month postpartum (±5 days), maternal bone lead was estimated by K x-ray fluorescence (K-XRF) at the research facility at the American British Cowdray Hospital, and anthropometric measurements, blood, and breast milk samples were obtained. Women who decided not to breastfeed their infants ($n = 12$) were excluded from the study. Subjects were then randomized to receive 1,200 mg of elemental calcium per day (as two 600-mg tablets of calcium carbonate, with the morning meal) or a placebo, starting the morning after the 1-month postpartum clinic visit (8).

All participants provided at least one breast milk sample, and no woman provided more than one milk sample at each time period. Breast milk samples were randomly chosen for analysis from among those who had information on other lead biomarkers (bone and blood). This report is limited to the 367 women with at least one laboratory-analyzed breast milk lead measurement available at any stage of the study (1, 4, and 7 months postpartum). Women with and without breast milk lead measurements were not different with respect to other lead biomarkers at baseline (6). Subjects were described as ''exclusive'' breastfeeding if, up to the time of the milk sample collection, they fed their infants with only breast milk. Mothers who supplemented their infants' feeding, combining breast milk with formula or other foods or liquids for infants, were described as ''partial'' breastfeeding.

Lead measurements

Blood lead measurements were performed with a model 3000 graphite furnace atomic absorption spectrophotometer (Perkin-Elmer, Wellesley, Massachusetts) at the American British Cowdray Hospital Trace Metal Laboratory according to a technique described by Miller et al. (10). The laboratory participates in the Centers for Disease Control and Prevention blood lead proficiency testing program and maintained acceptable precision and accuracy over the study period (correlation = 0.98; mean difference = 0.71 μg/dl; standard deviation (SD) = 0.68).

A spot-source cadmium-109 K-XRF instrument was used to measure maternal bone lead with 32 in vivo measurements of each subject's midtibial shaft (representing cortical bone) and patella (trabecular bone). The physical principles, technical specifications, validation, and use of the K-XRF technique have been described in detail elsewhere (11, 12). For purposes of quality control, we excluded bone lead measurements with uncertainty estimates of greater than 10 and 15 μg of lead per gram of mineral bone for tibia ($n = 12$) and patella ($n = 38$), respectively, from the original cohort of 629 women.

Breast milk samples were obtained and analyzed with techniques to minimize the potential for environmental contamination and to determine the lead concentrations in breast milk with a high percentage of recovery. Breast milk sample preparation was performed at the University Research Institute for Analytical Chemistry (Amherst, Massachusetts) by use of nitric acid digestion in a high-temperature, high-pressure asher (Anton Paar USA, Ashland, Virginia). The lead content of the samples was analyzed by isotope-dilution, inductively coupled, plasma mass spectrometry (Sciex Elan 5000; Perkin Elmer, Norwalk, Connecticut) with a limit of detection of 0.1 ng/ml at the Trace Metals Laboratory of the Harvard School of Public Health (Boston, Massachusetts).

Statistical analysis

Univariate and bivariate summary statistics and distributional plots were examined for all variables. Breast milk lead levels were highly positively skewed. Extreme outliers ($n = 10$) were identified by use of the generalized extreme studentized deviate many-outlier procedure (13) and excluded from multivariate analyses. The Spearman tests of correlation were used, and correlation coefficients with two-sided p values were reported. Summary statistics for breast milk lead at each stage of lactation stratified by maternal characteristics were calculated.

The associations of bone lead and blood lead with breast milk lead over the course of lactation were estimated by use

TABLE 1. Summary statistics for lead biomarkers among those subjects with breast milk lead levels available at 1, 4, and 7 months postpartum ($n = 367$), Mexico City, Mexico, 1994–1995

Biomarker of lead exposure	No.	Mean	Standard deviation	Median	Minimum	Maximum
Delivery						
Maternal blood lead (μg/dl)	361	8.6	41	7.8	2.1	23.7
Umbilical cord lead (μg/dl)	321	6.6	3.5	5.8	1.2	26.3
1 month postpartum						
Breast milk lead (μg/liter)	310	1.4	1.1	1.0	0.2	8.0
Maternal blood lead (μg/dl)	367	9.3	4.5	8.3	1.8	29.9
Maternal patella lead (μg/g)*	349	14.6	14.6	14.0	<1	67.2
Maternal tibia lead (μg/g)*	359	9.5	9.9	9.0	<1	76.5
4 months postpartum						
Breast milk lead (μg/liter)	224	1.2	1.0	0.9	0.2	6.8
Maternal blood lead (μg/dl)	340	9.0	4.0	8.2	2.0	26.7
7 months postpartum						
Breast milk lead (μg/liter)	195	0.9	0.8	0.7	0.2	4.8
Maternal blood lead (μg/dl)	320	8.1	3.4	7.4	2.1	23.2

* Includes measurements with negative values: patella ($n = 51$); tibia ($n = 52$).

of mixed-effects models with robust variance estimation and unstructured covariance. The log (base e)-transformed values of the dependent variable were used. Mixed-effects models take into account the correlation between repeated measures on subjects over time and allow us to examine heterogeneity in response to exposure by explicitly identifying individual (random effects) and population (fixed effects) characteristics. In addition, mixed models are flexible with respect to imbalance in the data. Subjects with incomplete data across time were included in order to increase the power of the study. Two separate analyses were performed: those confined to the subset of women with all three measurements during the study period ($n = 147$) and those including all subjects with at least one measurement ($n = 367$). Since the results were very similar, only the latter are presented. Separate models were estimated for each maternal lead biomarker (patella, tibia, blood), with a random intercept (subject) term and adjustment for the following fixed effects: period of study (number of months postpartum), calcium supplement group assignment (calcium vs. placebo), breastfeeding practice (exclusive vs. partial), dietary calcium intake (<1,000 or ≥1,000 mg) (dichotomized at the recommended dietary intake for lactating women (aged 19–50 years) (14)), primiparity (yes/no), history of previous lactation (≥12 months, yes/no), anemia status (hemoglobin, <13 g/dl (cutoff value adjusted for high altitude) (15), yes/no), and current use of lead-glazed ceramics (yes/no). Breastfeeding practice (exclusive vs. partial) was used as a proxy for the intensity of lactation. Parity and previous lactation history were included to account for prior periods of potentially accelerated bone lead mobilization. Residual plots and influence diagnostics (Cook's D statistic) were examined to ensure that influential observations were not driving the parameter estimates.

Based on the underlying suspected biologic mechanisms for the relations among bone, blood, and breast milk lead levels, interaction terms were developed to better characterize the effects of breastfeeding practice, calcium intake, and current use of lead-glazed ceramics (as a source of dietary lead intake) as potential modifiers of these associations over time. The individual heterogeneity of response was explored by next including a random slope component for each maternal lead exposure biomarker (in separate models). To explore the potential nonlinear associations between bone and breast milk lead levels, we used generalized additive models with a smooth term for the lead exposure variable (span = 0.75). All statistical analyses were performed using SAS, release 8.01, software (SAS Institute, Inc., Cary, North Carolina) and S-PLUS, version 6.0 Professional Edition for Windows, software (Insightful Corp., Seattle, Washington).

RESULTS

The number of subjects with breast milk lead levels and percentage reporting exclusive breastfeeding at each stage of the study were as follows: 1 month ($n = 310$, 32 percent), 4 months ($n = 224$, 9 percent), and 7 months ($n = 195$, 0 percent). There were 367 women with at least one breast milk lead measurement over the course of the study, 215 women with at least two measurements, and 147 women with all three measurements.

At 1, 4, and 7 months postpartum, respectively, the mean concentrations of breast milk lead were 1.4 (SD, 1.1), 1.2 (SD, 1.0), and 0.9 (SD, 0.8) μg/liter (table 1) and showed a significantly decreasing trend over the course of lactation ($p < 0.0001$). Maternal blood lead levels ranged from a minimum of 1.8 μg/liter to a maximum of 29.9 μg/dl and also decreased over the course of lactation ($p = 0.08$).

The levels of lead in breast milk at 1 month postpartum were significantly correlated with breast milk lead at 4 months ($r = 0.21$, $p = 0.006$) and 7 months ($r = 0.29$, $p = 0.0003$) postpartum. The levels at 4 and 7 months postpartum

TABLE 2. Summary statistics for breast milk lead at each stage of lactation, stratified by reported subject characteristics ($n = 367$), Mexico City, Mexico, 1994–1995

Variable by no. of months postpartum	No.	%	Breast milk lead (μg/liter)		*p* value*
			Mean	Standard deviation	
Age					
1 month postpartum					
<20 years	51	16	1.28	0.88	0.19
20–30 years	220	71	1.44	1.19	
>30 years	39	13	1.11	0.65	
Breastfeeding practice					
1 month postpartum					
Exclusive†	99	32	1.38	1.10	0.94
Partial‡	211	68	1.37	1.10	
4 months postpartum					
Exclusive	20	9	1.47	1.41	0.29
Partial	204	91	1.12	0.89	
7 months postpartum§					
Partial	195	100	0.94	0.75	
Previous pregnancies					
1 month postpartum					
Yes	181	58	1.39	1.08	0.73
No	129	42	1.34	1.12	
History of breastfeeding					
1 month postpartum					
Yes	104	34	1.36	1.03	0.87
No	206	66	1.38	1.22	
Current use of lead-glazed ceramics					
1 month postpartum					
Yes	126	41	1.53	1.17	0.04
No	184	59	1.26	1.03	
4 months postpartum					
Yes	64	29	1.24	0.76	0.33
No	160	71	1.11	1.01	
7 months postpartum					
Yes	32	16	0.98		0.76
No	163	84	0.93		
Past use of lead-glazed ceramics					
1 month postpartum					
Yes	236	76	1.40	1.11	0.39
No	74	24	1.27	1.05	
Dietary calcium intake¶					
1 month postpartum					
<1,000 mg	170	55	1.32	1.07	0.41
≥1,000 mg	140	45	1.42	1.12	
4 months postpartum					
<1,000 mg	150	67	1.13	0.85	0.64
≥1,000 mg	74	33	1.19	1.13	
7 months postpartum					
<1,000 mg	129	66	0.94	0.74	0.99
≥1,000 mg	66	34	0.94	0.78	
Calcium supplement group					
1 month postpartum#					
Yes	81	26	1.71	1.40	0.006
No	222	72	1.24	0.94	
4 months postpartum					
Yes	68	30	1.23	1.00	0.40
No	154	69	1.11	0.93	
7 months postpartum					
Yes	72	37	0.90	0.64	0.59
No	121	62	0.96	0.81	

* Two-sided *t* test for equality of sample population means.

† "Exclusive breastfeeding" was defined as nothing but breast milk used as the infant's feeding source.

‡ "Partial breastfeeding" was defined as breast milk plus formula and/or other liquid and solid food sources.

§ No women reported exclusive breastfeeding at 7 months postpartum.

¶ Dietary calcium was dichotomized at the US recommended dietary allowance for lactating women aged 19–50 years.

Represents the baseline difference in breast milk lead between the groups assigned to calcium or placebo.

Table continues

were also significantly correlated with each other ($r = 0.30$, $p < 0.0001$). The correlation of breast milk with concurrent blood lead level decreased over time ($r = 0.42$, 0.33, and 0.30; all $p < 0.0001$). Maternal bone lead levels (patella and tibia) were more highly correlated with blood lead ($r = 0.3$, $p < 0.0001$, and $r = 0.2$, $p = 0.001$, respectively) than with breast milk lead ($r = 0.2$, $p = 0.01$, and $r = 0.01$, $p = 0.83$, respectively) at 1 month postpartum.

Summary statistics for breast milk lead at each stage of lactation stratified by maternal characteristics are presented in table 2. Breast milk lead levels were not significantly different by maternal age, breastfeeding practice, primiparity, history of previous lactation, past use of lead-glazed ceramics, or dietary calcium intake. Current use of lead-glazed ceramics was associated with higher breast milk lead levels at 1 month postpartum ($p = 0.04$). However, by 4 and 7 months postpartum, no significant differences remained. As part of the study, women were counseled about the dangers of using lead-glazed ceramics, and the percentage of use declined over the course of the study from 41 percent at baseline to 16 percent at 7 months postpartum. When examined by calcium supplement group assignment,

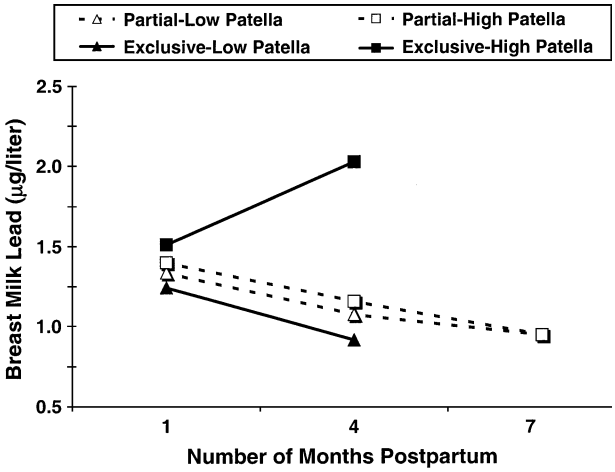

FIGURE 1. Mean breast milk lead levels (μg/liter) over the course of lactation (1, 4, and 7 months postpartum), by breastfeeding practice and patella lead level (dichotomized at the median), Mexico City, Mexico, 1994–1995. Exclusive breastfeeding is indicated by solid lines, and partial breastfeeding is indicated by dashed lines.

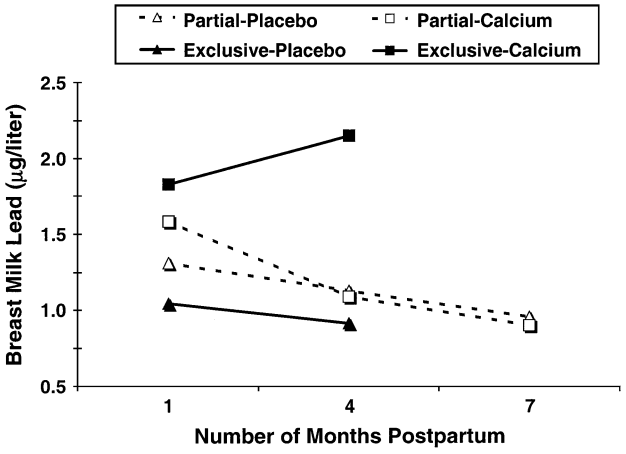

FIGURE 2. Mean breast milk lead levels (μg/liter) over the course of lactation (1, 4, and 7 months postpartum), by breastfeeding practice and calcium supplement group assignment, Mexico City, Mexico, 1994–1995. Exclusive breastfeeding is indicated by solid lines, and partial breastfeeding is indicated by dashed lines.

women in the calcium group had higher breast milk lead levels at baseline ($p = 0.006$). However, this was not related to the supplement itself since initiation of calcium (or placebo) occurred after baseline measurements were obtained.

Figures 1, 2, and 3 explore potential interactions and show the unadjusted effects of exclusive lactation on breast milk lead levels. On average, breast milk lead declined over the course of lactation. However, among women practicing exclusive lactation, those with high levels of patella lead (figure 1), assigned to the calcium supplement group (figure 2), or currently using lead-glazed ceramics (figure 3) tended to have breast milk lead levels that increased over the course of lactation.

In a model with a random intercept term, adjustment for the period of study, calcium supplement group assignment, breastfeeding practice, dietary calcium intake, primiparity, history of previous lactation, anemia status, or current use of lead-glazed ceramics (table 3), patella lead was a significant predictor of breast milk lead over the course of lactation. An interquartile-range increase in patella lead was associated with a 10 percent increase in breast milk lead (95 percent confidence interval: 2, 18), while an interquartile-range increase in tibia lead was associated with a 4 percent increase in breast milk lead (95 percent confidence interval: −2, 10). Maternal blood lead was the strongest predictor of breast milk lead, with an interquartile-range increase associated with a 36 percent increase in breast milk lead concentration (95 percent confidence interval: 29, 44). Dietary calcium supplementation increased the rate of decline in breast milk lead by 5–10 percent over the course of lactation in comparison with placebo (table 4). The effect was highest in the models for bone lead (patella, 10 percent; tibia, 8 percent) and lowest in the models for blood lead (5 percent). Interaction terms for breastfeeding practice and supplement group were statistically significant for the effect of patella ($p = 0.004$) and tibia ($p = 0.004$) lead on breast milk lead. This is consistent with the hypothesis that intensity of lactation is a modifier of the relation between cumulative lead exposure (estimated by bone lead) and breast milk lead.

The individual heterogeneity of response was explored by using models with a random slope for each maternal lead exposure biomarker (in separate models), and histograms of the individual slopes for each lead biomarker are shown in figures 4, 5, and 6. Individual slopes (fixed plus random effect) for blood ranged from 0.045 to 0.070, suggesting that there was substantial between-subject variation in breast milk lead for a given blood lead measurement. Subjects showed less

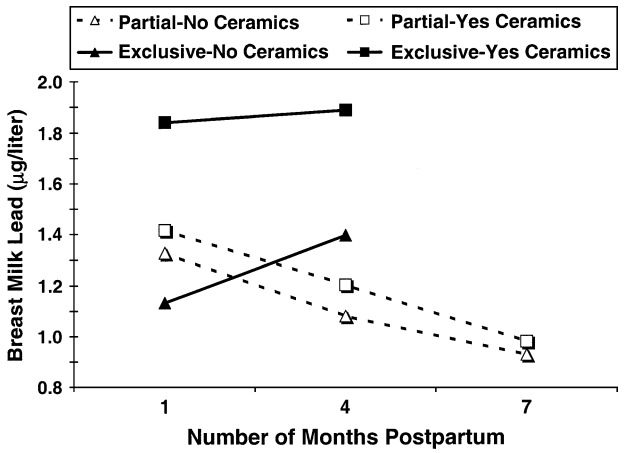

FIGURE 3. Mean breast milk lead levels (μg/liter) over the course of lactation (1, 4, and 7 months postpartum), by breastfeeding practice and current use of lead-glazed ceramics, Mexico City, Mexico, 1994–1995. Exclusive breastfeeding is indicated by solid lines, and partial breastfeeding is indicated by dashed lines.

TABLE 3. Repeated-measures analysis of breast milk lead* levels at 1, 4, and 7 months postpartum among women ($n = 367$), by use of a random intercept model, Mexico City, Mexico, 1994–1995

Variable	Model for patella		Model for tibia		Model for blood	
	Parameter estimate	95% confidence interval	Parameter estimate	95% confidence interval	Parameter estimate	95% confidence interval
Intercept	1.07	0.86, 1.33	1.14	0.92, 1.41	0.69	0.57, 0.85
Period of study†						
4 months	0.83	0.73, 0.94	0.83	0.73, 0.94	0.82	0.73, 0.93
7 months	0.69	0.60, 0.79	0.68	0.59, 0.78	0.69	0.60, 0.79
Calcium supplement group‡	1.04	0.91, 1.20	1.04	0.91, 1.20	1.09	0.95, 1.24
Breastfeeding practice§	1.09	0.95, 1.26	1.07	0.93, 1.22	1.02	0.90, 1.15
Dietary calcium, ≥1,000 mg/day (yes)	0.94	0.84, 1.04	0.95	0.86, 1.06	0.96	0.87, 1.05
Primiparity (yes)	0.91	0.77, 1.08	0.90	0.76, 1.06	0.87	0.75, 1.01
History of lactation (≥12 months)	0.94	0.84, 1.06	0.94	0.84, 1.05	0.94	0.86, 1.04
Anemia (Hg, <13 g/dl)¶	1.01	0.88, 1.15	1.00	0.87, 1.14	1.01	0.90, 1.13
Current use of lead-glazed ceramics (yes)	1.15	1.04, 1.27	1.15	1.03, 1.27	1.03	0.94, 1.13
Maternal lead biomarker#	1.10	1.02, 1.18	1.04	0.98, 1.10	1.36	1.29, 1.44

* Breast milk lead levels log (base *e*) transformed; parameter estimate = antilog of the β coefficient from the model.
† Referent category: 1 month postpartum.
‡ 1 = calcium supplement, and 0 = placebo.
§ Referent category: partial lactation.
¶ Anemia cutoff values were adjusted for altitude (7,000–7,999 feet (2,133.6–2,438.1 m) above sea level) (15).
# Calculated for an interquartile-range increase (patella = 20 μg/g; tibia = 12 μg/g; blood = 5 μg/dl) in lead biomarker.

variability in the individual response slopes for patella lead (from 0.001 to 0.01) and for tibia lead (from –0.01 to 0.007).

Figures 7, 8, and 9 show the adjusted dose-response curves for the natural log-tranformed breast milk lead levels for each maternal biomarker from generalized additive models with a random subject term. After adjustment for period of study (number of months postpartum), calcium supplement group assignment, breastfeeding practices, dietary calcium intake, primiparity, history of previous lactation, maternal hemoglobin level, and current use of lead-glazed ceramics, the maternal blood lead biomarker shows a fairly linear positive association with breast milk lead levels. The analysis using generalized additive models suggested a nonlinear relation with patella lead and tibia lead and the log-scaled breast milk lead levels. To account for this pattern, we included both a quadratic term and a cubic term in the regression models (separately), but this made virtually no difference in the coefficients (data not shown). This relation was also explored by categorizing bone lead into quintiles. Since the coefficients remained similar to the model assuming linearity, the results from the most parsimonious model are presented.

TABLE 4. Effect of calcium supplementation on changes in breast milk lead* levels over the course of lactation, Mexico City, Mexico, 1994–1995

	4 months postpartum		7 months postpartum	
	% of baseline	% change	% of baseline	% change
Patella				
Calcium	79	−21	62	−38
Placebo	85	−15	72	−28
Difference (%)		6		10
Tibia				
Calcium	80	−20	64	−36
Placebo	85	−15	72	−28
Difference (%)		5		8
Blood				
Calcium	80	−20	65	−35
Placebo	84	−16	70	−30
Difference (%)		4		5

* Adjusted for breastfeeding practice, dietary calcium intake, primiparity, history of previous lactation, anemia status, and current use of lead-glazed ceramics.

DISCUSSION

Our study provides direct evidence that the amount of lead released from bone is related to lactation intensity and, subsequently, to levels of lead in breast milk. In general, breast milk lead levels declined over the course of lactation. However, among those women practicing exclusive lactation and 1) those with high patella lead, 2) those assigned to the calcium supplement group, or 3) those who were current users of lead-glazed ceramics, breast milk lead levels tended to increase over the course of lactation. This indicates that breastfeeding practice (exclusive lactation) is an important effect modifier over time.

Other studies have shown similarly consistent findings. In a study of community volunteers, Kosnett et al. (16) found

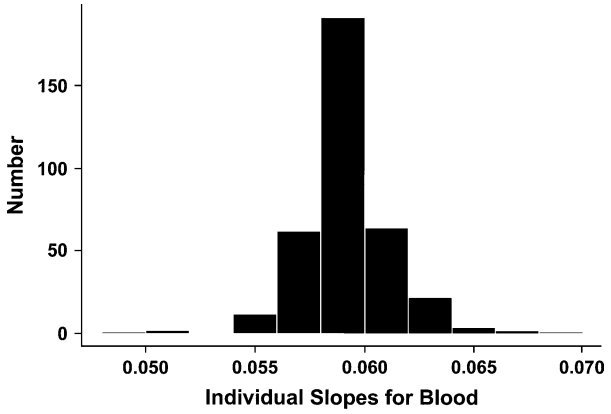

FIGURE 4. Individual slopes (fixed plus random effects) for the effect of maternal blood lead on breast milk lead from mixed-effects models, Mexico City, Mexico, 1994–1995.

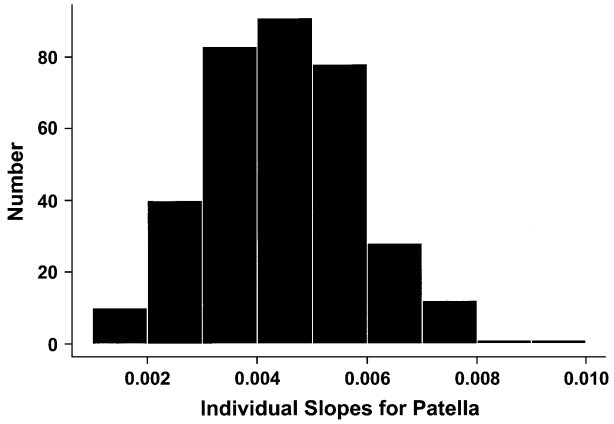

FIGURE 5. Individual slopes (fixed plus random effects) for the effect of maternal patella lead on breast milk lead from mixed-effects models, Mexico City, Mexico, 1994–1995.

that women with a history of breastfeeding had lower age-adjusted tibia bone lead levels than those without a history of lactation, suggesting that lactation depletes maternal bone lead. Researchers have also noted a significant inverse relation between months of lactation and age-adjusted calcaneus lead among 24 lactating women in Mexico City (17). Osterloh and Kelly (18) assessed lactating women prospectively to study the effect of bone loss on blood lead concentrations and found that bone density losses averaged 2.5 percent at the vertebral spine and almost 1 percent at the femoral neck. The total number of breastfeedings was the only significant independent predictor of final bone density apart from the initial bone density.

The results presented here are consistent with the results of our previous study that examined the impact of breastfeeding practices on mobilization of lead from bone. Women with high bone lead levels and a high intensity of breastfeeding mobilized more lead than did those with lower lead burdens and less intensive breastfeeding practices, as reflected by their blood lead levels over the course of lactation (1). However, exclusive breastfeeding rates and duration in Mexico City are low (19). Only 32 percent of subjects who provided breast milk samples reported exclusive breastfeeding at 1 month postpartum, and only 9 percent were breastfeeding exclusively at 4 months postpartum. By 7 months postpartum, no subjects were exclusively breastfeeding their infants, so it is difficult to draw conclusions about the influence of breastfeeding practice on breast milk lead levels over the course of lactation.

The subjects in our study included only a subset of the original randomized placebo-controlled trial of calcium supplementation during lactation and, in our convenience sample of subjects who provided breast milk, the levels of breast milk lead at baseline were higher in the calcium supplement group ($p = 0.006$). Nonetheless, levels of breast milk lead in the calcium supplement group declined at a faster rate than did those in the placebo group, so that there were no statistically significant differences in breast milk lead between the two groups at the end of the study. In stratified analyses, it appeared that, among those women practicing exclusive lactation and assigned to the calcium supplement group, breast milk lead levels tended to increase over the course of lactation. In fully adjusted models, however, calcium supplementation increased the rate of decline in breast milk lead by 5–10 percent over the course of lactation, in comparison with placebo, suggesting that calcium supplementation may be an important potential intervention strategy to reduce lead in breast milk from both current and previously accumulated sources. The effect was highest in the models for bone lead (8–10 percent) and lowest in the models for blood lead (5 percent).

Dietary factors are known to have a significant impact on lead dynamics, particularly with respect to the absorption of lead from the gastrointestinal tract (20–22). Lead competes with calcium at calcium-binding sites and may subsequently

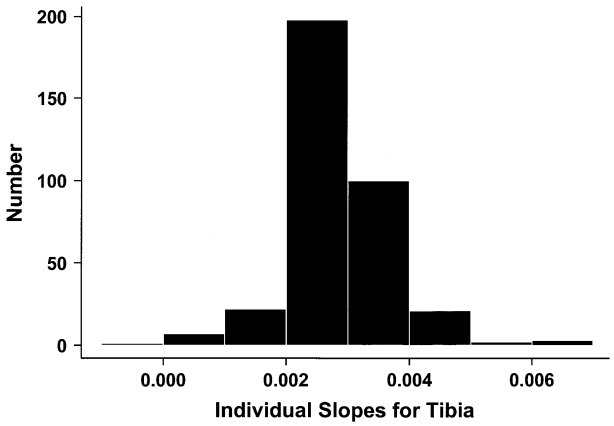

FIGURE 6. Individual slopes (fixed plus random effects) for the effect of maternal tibia lead on breast milk lead from mixed-effects models, Mexico City, Mexico, 1994–1995.

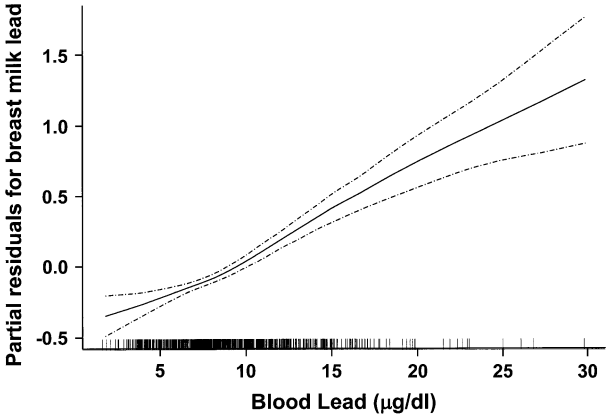

FIGURE 7. Adjusted dose-response curves for log (base *e*)-transformed breast milk lead levels for maternal blood lead from a generalized additive model with a random subject term, Mexico City, Mexico, 1994–1995. Curves were adjusted for the period of study (number of months postpartum), calcium supplement group, breastfeeding practices, dietary calcium intake (mg), primiparity, history of previous lactation (≥12 months), maternal hemoglobin (g/dl), current use of lead-glazed ceramics, and a smoothed term for maternal lead biomarker (span = 0.75). Dashed lines represent confidence intervals.

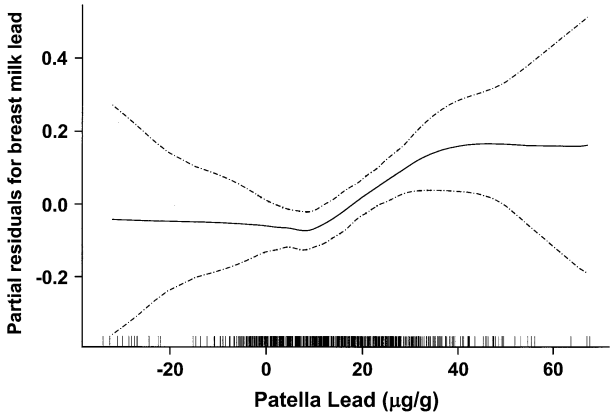

FIGURE 8. Adjusted dose-response curves for log (base *e*)-transformed breast milk lead levels for maternal patella lead from a generalized additive model with a random subject term, Mexico City, Mexico, 1994–1995. Curves were adjusted for the period of study (number of months postpartum), calcium supplement group, breastfeeding practices, dietary calcium intake (mg), primiparity, history of previous lactation (≥12 months), maternal hemoglobin (g/dl), current use of lead-glazed ceramics, and a smoothed term for maternal lead biomarker (span = 0.75). Dashed lines represent confidence intervals.

alter protein function and calcium homeostatis (23). Calcium deficiency has been shown to increase lead absorption (24) and lead retention (25).

The factors controlling skeletal changes of pregnancy and lactation are still unknown. Lactation has discernible effects on calcium homeostasis that requires a substantial redistribution of calcium from maternal bone stores to meet the needs of the nursing infant (26, 27). Bone loss observed during lactation appears to be transient, with levels returning to baseline after the return of ovarian function and cessation of nursing (28). Calcium supplementation has been shown to have little effect on lactation-induced changes in bone turnover (29, 30). However, baseline dietary intake and levels of calcium supplementation in recent studies have been relatively low. It is possible that very high levels of calcium are needed to counterbalance the nutritional needs of the developing fetus (31). Other genetic, hormonal, or lifestyle factors may also be responsible.

A limitation of the current study is that there was no information available on bone mineral density or biomarkers of bone remodeling, so the hypothesis that lactation increases bone mobilization, and thus breast milk lead levels, could not be evaluated directly. Using quantitative markers of bone mineral density and bone resorption, Sowers et al. (32) found a positive association between maternal bone loss and change in breast milk lead concentration ($r = 0.51$, $p < 0.001$). Our study had the advantage of comparing multiple lead biomarkers over time among a large number of women with relatively high cumulative lifetime exposures to lead. We were able to show that relations between bone and blood to breast milk lead were modified by breastfeeding practice, with the highest breast milk lead levels among women with high levels of patella lead who were exclusively breastfeeding. The main environmental source of lead, use of lead-glazed ceramics for preparing and storing foods, was accounted for in the analysis so that changes in breast milk lead could not be explained by dietary lead alone.

Data from the current study suggest that, despite the potential for lead exposure, levels of lead in breast milk over the course of lactation are low. However, the decline of environmental sources of lead highlights the relevance of

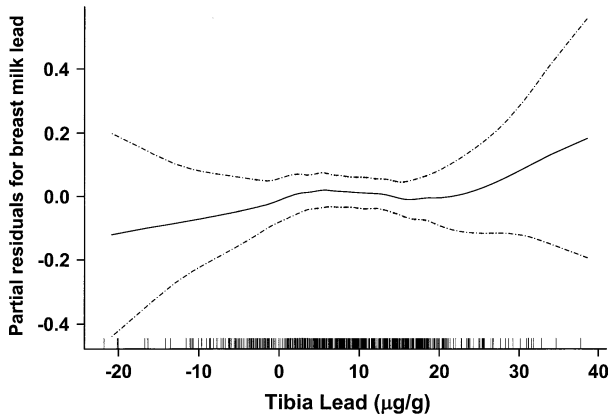

FIGURE 9. Adjusted dose-response curves for log (base *e*)-transformed breast milk lead levels for maternal tibia lead from a generalized additive model with a random subject term, Mexico City, Mexico, 1994–1995. Curves were adjusted for the period of study (number of months postpartum), calcium supplement group, breastfeeding practices, dietary calcium intake (mg), primiparity, history of previous lactation (≥12 months), maternal hemoglobin (g/dl), current use of lead-glazed ceramics, and a smoothed term for maternal lead biomarker (span = 0.75). Dashed lines represent confidence intervals.

maternal bone as a continuing endogenous source of exposure. Since bone lead has a half life of years to decades, women and their infants will continue to be at risk for exposure long after environmental sources of lead have abated. In addition to reducing ongoing exposure to lead from exogenous sources, such as diet, this highlights the need to further investigate low-cost interventions, such as calcium supplementation, which may reduce lead exposure from previously accumulated, endogenous sources. Nutritional intervention may be an important strategy for preventing transgenerational exposures from lead-exposed women during the reproductive years.

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