Dietary Calcium Supplements to Lower Blood Lead Levels in Lactating Women: A Randomized Placebo-Controlled Trial

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Background. Pregnancy and breastfeeding mobilize lead stored in bone, which may be a hazard for the fetus and infant. We tested the hypothesis that in lactating women a dietary calcium supplement will lower blood lead levels.

Methods. Between 1994 and 1995 we conducted a randomized trial among women in Mexico City. Lactating women (N = 617; mean age = 24 years; mean blood lead level = 8.5 ug/dL) were randomly assigned to receive either calcium carbonate (1200 mg of elemental calcium daily) or placebo in a double-blind trial. Blood samples were obtained at baseline, and 3 and 6 months after the trial began. Blood lead was determined by graphite furnace atomic absorption spectroscopy. Bone lead was measured at baseline with a 109cd K x-ray fluorescence instrument. The primary endpoint was change in maternal blood lead level, which was analyzed in relation to supplement use and other covariates by multivariate generalized linear models for longitudinal observations.

Results. An intention-to-treat analysis showed that women randomized to the calcium supplements experienced a small decline in blood lead levels (overall reduction of 0.29 ug/dL; 95% confidence interval = −0.85 to 0.26). The effect was more apparent among women who were compliant with supplement use and had high bone lead levels (patella bone lead ≥5 μg/gm bone). Among this subgroup, supplement use was associated with an estimated reduction in mean blood lead of 1.16 ug/dL (95% confidence interval = −2.08 to −0.23), an overall reduction of 16.4%.

Conclusions. Among lactating women with relatively high lead burden, calcium supplementation was associated with a modest reduction in blood lead levels.

(EPIDEMIOLOGY 2003;14:206–212)

Key words: randomized clinical trial, breastfeeding, blood lead, calcium supplementation.

Effective prevention of lead exposure for fetuses and breastfeeding infants requires identification and control of sources of environmental lead exposure for pregnant women, as well as control of endogenous maternal bone lead stores.[1–3] In the adult, 95% of lead accumulates in bone.[4] With a half-life of decades,[5] bone lead levels remain elevated despite declines in blood lead. Pregnancy and lactation are known to be associated with a marked increase in maternal bone turnover,[1] which may augment mobilization of lead from bone stores.[6–8] Thus, lactation places women with high bone lead burdens, and also their breastfed infants, at an increased risk of lead exposure from endogenous sources.[9] Some studies[10–11] have shown that calcium supplementation may decrease bone loss during lactation. These findings suggest that increasing maternal calcium consumption through dietary supplementation, particularly in women with relatively low levels of dietary calcium, could reduce bone resorption and therefore bone lead release.

Although evidence from experimental[11–15] and observational studies[16–17] suggests that increasing dietary calcium may be a cost-effective intervention for decreasing

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This study was supported by the U.S. NIEHS P42-ES05947 (with support provided by the EPA), NIEHS R01ES07821, and NIEHS Center Grant 2 P30 ES 00002; Consejo Nacional de Ciencia y Tecnologia (CONACyT) Grant 4150M9405; and CONSERVA, Department of the Federal District, Mexico.

Submitted 12 November 2001; final version accepted 6 September 2002.

fetal lead exposure, no reports in the literature have specifically evaluated this hypothesis in the context of a randomized clinical trial. To address this issue, we conducted a double-blind randomized clinical trial in lactating women living in Mexico to test the hypothesis that women taking a calcium supplement (1200 gm per day) will have lower venous blood lead levels than women taking a placebo. We also tested whether the supplement effect was modified by endogenous lead sources. We chose women in Mexico City because dietary calcium intake is low and the use of calcium supplements during lactation is not common. Also, Mexico City has recently phased lead out of gasoline, thereby accentuating bone lead stores as an endogenous source lead exposure.

Methods

Sample Selection

Between January 1994 and June 1995, we interviewed 2910 women admitted for labor and delivery; of these, 1382 were eligible for the study. Study methods have been described elsewhere.[9] Briefly, inclusion criteria included intention to breastfeed, residency in Mexico City and a normal pregnancy and delivery. Baseline information and umbilical cord and maternal venous blood specimens were obtained at delivery from all eligible participants. One month (±5 days) after delivery, participants were invited to attend our research center. Of the 1382 eligible women identified in the first interview, 629 (44.6%) agreed to participate in the trial and completed an evaluation that included a questionnaire to assess known risk factors for environmental lead exposure,[18] a food-frequency questionnaire to assess dietary calcium intake,[19] and a physical exam that included anthropometry and lead measurements in blood, breast milk and bone. We excluded women who had stopped breastfeeding (N = 12). The remaining 617 (all breastfeeding) were randomly assigned to receive 1200 gm of elemental calcium per day in the form of calcium carbonate (N = 296) or a placebo (N = 321). The calcium supplement was provided as two 600-mg tablets, and women were instructed to consume the tablets with their morning meal. The placebo and supplement were prepared by Lederle, Inc. (Mexico), had an identical appearance and were free of any taste or aftertaste. Compliance was assessed by pill count every 3 months.

Blood and breast milk samples were obtained 3 and 6 months after calcium supplementation began; the breast milk results are not yet available. At 3 months participants were visited at home by field personnel who obtained biological samples and updated questionnaire information. They also collected bottles with remaining pills and gave the participants a second set of calcium supplement or placebo for the next 3 months. At 6 months participants attended the research center, where information was updated, blood and breast milk samples were collected and a second bone lead measurement was obtained.

The research protocol was approved by the Human Subjects Committee of the National Institute of Public Health of Mexico. All participants gave their informed consent and received a detailed explanation of the study and procedures used, as well as counseling on how to reduce their lead exposure.

Lead Measurements

Blood samples were analyzed with a graphite furnace atomic absorption spectrophotometry instrument (Perkin Elmer 3000) at the metals laboratory of the American British Cowdray (ABC) Hospital in Mexico City. This laboratory complied with the standardization program of the Wisconsin State Laboratory of Hygiene in Madison, WI.

Bone lead measurements were taken of each subject's mid-tibial shaft (cortical bone) and patella (trabecular) using a spot-source 109cd K x-ray fluorescence instrument. The physical principles, technical specifications, validation and use of this and other K x-ray fluorescence instruments have been described in detail elsewhere.[20–21] For the present study, 30-minute measurements were taken at the mid-shaft of the left tibia (cortical bone) and at the left patella (trabecular bone).

Statistical Analyses

The primary outcome of interest, change in maternal blood lead levels, was evaluated by comparing blood lead levels measured at baseline (prerandomization) with those at 3 and 6 months after the supplement had first been ingested. Because we had multiple measurements of the outcome over time our analysis incorporated a longitudinal design. To estimate the effect of calcium supplement, we applied the following base model:

$$y_{ij} = \beta_0 + \beta_1 t_{3ij} + \beta_2 t_{6ij} + \beta_3 sup + \beta_4 sup t_{3ij} + \beta_5 sup t_{6ij} + \varepsilon_{ij} \quad (1)$$

where y_{ij} is the blood lead of the ith subject on the jth visit, t_3 and t_6 are indicator variables for visits at 3 and 6 months after the trial began, and sup is an indicator for whether the subject received the treatment ($sup = 1$) or placebo ($sup = 0$). The estimated coefficients for β_1 and β_2 represent the mean difference between baseline and the 3- and 6-month evaluation, β_3 represents the difference between the placebo and intervention groups at baseline, and β_4 and β_5 are the estimates of the treatment effect at 3 and 6 months.

Although the primary protection against confounding was the randomized design of our study, we included several covariates that were predictors of blood lead in

Study Population at different times of the trial	Trial	Intervention	Placebo
Women interviewed at postpartum	2910	-	-
Eligible to participate in the study	1382	-	-
Agreed to participate in the study	629	-	-
Excluded because no longer were breastfeeding	12	-	-
Randomized at 1 month postpartum	**617**	**296**	**321**
3 months of follow-up			
Excluded because of missing blood lead	57	26	31
Data available	**560**	**270**	**290**
6 months of follow-up			
Excluded because of missing blood lead	50	23	27
Data available	**510**	**247**	**263**

FIGURE 1. Summary of participants' enrollment, follow-up and eligibility analysis for changes in blood lead levels.

our multivariate models. We did so not only to prevent residual confounding, but also to increase statistical power. Regression models were extended to include determinants of blood lead such as bone lead, use of lead-glazed ceramics, breastfeeding and other reproductive variables. To test the hypothesis that the supplement effect was modified by bone lead levels, we estimated supplement effect among subgroups of participants with increasing mean bone lead.

All model parameters were estimated with generalized linear models (GLMs) for longitudinal observations[22–23] using Stata.

Results

Study participation flow is presented in Figure 1. Of the 617 participants who initiated the study, 83% (N = 510) completed the 6 months of follow-up. At 6 months, 32% of the original cohort continued to breastfeed (N = 197; 103 placebo and 94 intervention).

We found no meaningful differences in age, years of school, number of pregnancies and blood and bone lead levels between the women who completed the trial and those who discontinued participation (Table 1). The randomization produced intervention and placebo groups that were similar in baseline characteristics except for slightly higher patella bone lead levels in women in the intervention group (the observed difference was of 2.7 μg-Pb/gm bone; 95% Confidence Interval [CI] = 0.26 to 5.13 μg-Pb/gm bone). The proportions of women who took 50% of pills (according to the pill count) were similar in the intervention and placebo groups (88.9% and 91.1%, respectively).

Supplement use was associated with a modest decline of blood lead levels (Table 2). Compared with women who received the placebo, those who took supplements had a modest decrease of −0.12 μg/dL in their blood lead levels over the study period at 3 months (CI = −0.71 to 0.46 μg/dL) and −0.22 μg/dL at 6 months (−0.77 to 0.34 μg/dL). The effect was more apparent when we restricted the analyses to women who were adequately compliant with supplement use (defined as taking 50% or more of the pills, by pill count) and among women with high bone lead levels (Table 2). The estimated effect of the supplement was even more apparent among women who completed the 6 months of follow-up and had high patella lead levels (>5 μg/gm bone, corresponding to the 25th percentile). Among this subgroup, supplement use was associated with a decline in blood lead that exceeded that of the placebo users by a mean of 1.16 μg/dL (CI = 0.23 to 2.08

TABLE 1. Characteristics of Women Participating in a Randomized Trial of Calcium Supplements According to Their Distribution in Participation Groups*

	Enrolled in the Trial (N = 617)		Declined Participation After Enrollment (N = 107)	Interrupted Breastfeeding (N = 196)
	Calcium Group (N = 296)	Placebo Group (N = 321)		
Age in years	24.5 ± 5.1	24.4 ± 5.2	23.5 ± 4.5	24.0 ± 5.2
Number of years in school	9.4 ± 3.1	9.1 ± 3.2	9.5 ± 3.1	9.6 ± 2.9
Tap water not available in the household (%)	5	5	6	2
Height (cm)	153 ± 5.8	153 ± 5.2	153 ± 5.8	152 ± 5.3
Number of pregnancies†	1.9 ± 1.1	2.0 ± 1.3	1.87 ± 1.2	1.9 ± 1.2
First pregnancy (%)	43	44	49	54
Total caloric intake per day‡ (baseline)	2,336 ± 761	2,262 ± 708	2,149 ± 652	2,420 ± 750
Dietary calcium intake‡ (baseline)	1,160 ± 531	1,137 ± 597	1,031 ± 447	1,145 ± 665
Compliance ≥50%§	87	88	NA	NA
Use of lead-glazed ceramics at baseline (%)	34	35	32	30
Use of lead-glazed ceramics at 6 months after randomization (%)	14	18	NA	NA
Blood lead at baseline (μg/dL)	9.2 ± 4.2	9.4 ± 5.0	9.1 ± 5.3	NA
Tibia lead at baseline (μg/gm bone)	10.7 ± 9.8	9.6 ± 10.3	8.9 ± 8.8	9.3 ± 10.2
Patella lead at baseline (μg/gm bone)	16.2 ± 15.7	13.5 ± 15.1	14.0 ± 12.1	13.5 ± 16.2

NA = not applicable.
* Results given with plus-minus values are means ± SD.
† Including the pregnancy just past.
‡ Estimated at 1 month postpartum with a food-frequency questionnaire.
§ Adequate compliance is defined as taking 50% or more of pills, as assessed by pill count.

TABLE 2. Determinants of Blood-Lead Levels in the Study Population and Estimated Effects According to Treatment Group for Subgroups of the Study Population

Variables	Change Among Participants Who Completed at Least One Follow-Up Measurement (N = 617)		Change Among Participants Who Completed the 6 Months' Evaluation (N = 610)	
	Coefficient	95% CI	Coefficient	95% CI
Model constant (μg/dL)	7.56	6.94 to 8.18	7.29	6.60 to 8.00
History of breastfeeding*	−0.75	−1.29 to −0.21	−0.78	−1.37 to −0.19
Patella lead (μpb/μg bone mineral)	0.07	0.04 to 0.09	0.07	0.04 to 0.093
Use of lead ceramics†	1.56	1.04 to 2.08	1.89	1.26 to 2.51
Breastfeeding‡	0.70	0.25 to 1.14	0.84	0.27 to 1.40
Change at 3 months§	0.03	−0.44 to 0.51	NA	
Change at 6 months‖	−0.74	−1.23 to −0.26	−0.60	−1.09 to −0.10
Calcium effect at 3 months	−0.12	−0.71 to 0.46	NA	
Calcium effect at 6 months	−0.22	−0.77 to 0.34	−0.29	−0.85 to 0.26
Participants with high compliance¶				
Change at 3 months	−0.07	−0.69 to 0.55	NA	
Change at 6 months	−0.79	−1.44 to −0.14	−0.61	−1.29 to 0.078
Calcium effect at 3 months	−0.16	−0.91 to 0.59	NA	
Calcium effect at 6 months	−0.51	−1.24 to 0.22	−0.71	−1.46 to 0.028
Participants with high compliance and baseline patella lead >5 μg-Pb/gm bone mineral				
Change at 3 months	−0.15	−0.92 to 0.61	NA	
Change at 6 months	−0.55	−1.37 to 0.28	−0.26	−1.16 to 0.63
Calcium effect at 3 months	−0.05	−0.93 to 0.84	NA	
Calcium effect at 6 months	−0.90	−1.80 to 0.001	−1.16	−2.08 to −0.23
Participants with high compliance and baseline patella lead > 10 μg-Pb/gm bone mineral				
Change at 3 months	−0.25	−1.072 to 0.564	NA	
Change at 6 months	−0.49	−1.42 to 0.44	−0.16	−1.17 to 0.84
Calcium effect at 3 months	−0.19	−1.12 to 0.74	NA	
Calcium effect at 6 months	−1.18	−2.17 to −0.20	−1.46	−2.48 to −0.45
Participants with high compliance and baseline patella lead >15 μg-Pb/gm bone mineral				
Change at 3 months	−0.46	−1.39 to 0.48	NA	
Change at 6 months	−0.47	−1.58 to 0.64	−0.04	−1.23 to 1.16
Calcium effect at 3 months	−0.22	−1.26 to 0.82	NA	
Calcium effect at 6 months	−1.53	−2.65 to −0.42	−1.86	−3.01 to −0.71

NA = not applicable.
* Modeled as a fixed variable, reference category is no history of having breast fed.
† Modeled as a time-dependent variable, reference category is no use of lead-glazed ceramics.
‡ Modeled as a time-dependent variable, reference category is stopped breastfeeding.
§ Modeled as a time-dependent variable, reference category is blood-lead at baseline.
‖ Modeled as a time-dependent variable, reference category is blood-lead at baseline.
¶ Modeled as a time-dependent variable, defined as taking 50% or more of pills as assessed by pill count.

μg/dL). The effect associated with the supplement was stronger after 6 months of the supplement and among women who continued breastfeeding at the 6-month evaluation, who had adequate compliance with the intervention and who had higher bone lead levels (Figure 2).

Both patella and tibia lead were positively associated with blood lead levels. Patella lead increased blood lead levels by 0.067 μg/dL per μg of bone lead (CI = 0.044 to 0.090 μg/dL per μg of bone lead) and tibia by 0.066 μg/dL per μg of bone lead (0.0418 to 0.090 μg/dL per μg of bone lead). We tested for interactions between supplement use and bone lead levels. The interaction term for patella lead was statistically substantial. The estimated coefficients for the interaction terms were negative (−0.022 μg/dL per μg of bone lead [$P = 0.17$] and −0.034 μg/dL per μg of bone lead [$P = 0.03$] for the estimated effects at 3 and 6 months, respectively), which suggests that the supplement use attenuated the release of lead from bone and that the effect increased with increasing levels of patella lead.

To further explore the supplement effect dependence on bone lead levels, we estimated the effect of the supplement in strata defined by bone lead levels. For these analyses we used as cutoff points to define the strata the percentile values of the distribution of patella lead. Results are summarized in Figure 3. We observed that the protective effect of the supplement increased among women with a high lead burden. At the 6-month evaluation, women who had bone lead levels above the 10th percentile had a supplement-related reduction of 11% in their blood lead levels, whereas women with patella lead levels above the 80th percentile had a 21% decrease in their blood lead levels.

Although tibia lead was an important predictor of blood lead levels, there was no evidence that tibia lead modified the effect of the supplement (data not shown).

We also evaluated the corresponding longitudinal changes in bone lead concentration associated with breastfeeding and the supplement. In comparison with women who stopped breastfeeding during the study period, those who continued and were randomized to the placebo group had a substantial decrease in patella lead concentrations (2.50 μg/gm lower bone levels; CI = 0.08 to 4.92). In contrast, women who continued breast-

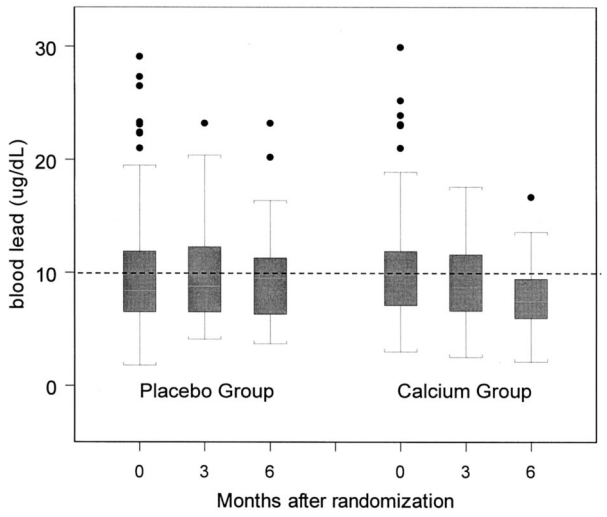

FIGURE 2. Blood lead levels for placebo and calcium groups according to stage of the study, for women with adequate compliance (pill counts ≥50%) who breastfed for 7 months and who had high bone lead levels (>5 μg-Pb/gm bone mineral). Blood lead decline over the 6-month period was 0.3 μg/dL ($P = 0.63$) and 2.3 μg/dL ($P < 0.01$) for placebo and calcium groups, respectively. Box plots show the median, quartiles as well as possible outliers (points further than 1.5 = interquartile ranges).

feeding but were randomized to the calcium group had only a small change in patella lead levels (mean difference of 0.50 μg/gm of bone; CI = −5.15 to 3.98).

Discussion

In this randomized clinical trial, assignment to calcium supplementation (1200 mg per day) was associated with a small decline of blood lead levels among lactating women. When the analyses were stratified according to compliance and restricted to women who breastfed for 6 months and who had higher trabecular bone lead levels, we observed a greater reduction in blood lead levels. This latter observation gives support to the hypothesis that the calcium supplements may have exerted this effect by reducing release of lead from bone rather than by decreasing lead absorption from the gastrointestinal track.

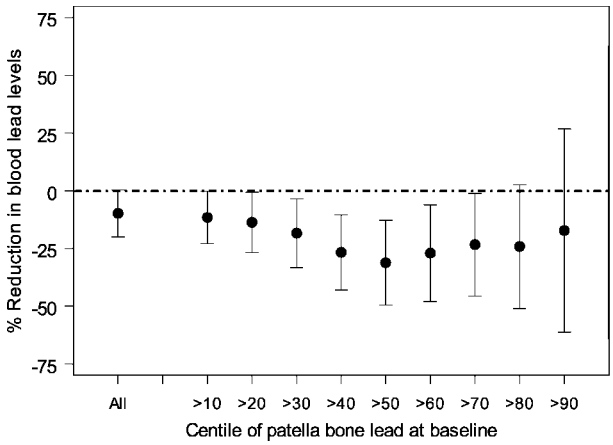

FIGURE 3. Reduction in blood-lead levels associated with calcium supplementation among subgroups of the study population with varying bone lead burdens at baseline. Effects are estimated for participants who had adequate compliance rates (pill counts ≥50%) and completed 6 months of follow-up. Subgroups were defined, including all women with patella bone lead levels above the indicated decile of bone lead. *Vertical lines* indicate the 95% CI.

Our findings are consistent with results derived from cross-sectional epidemiologic studies that have addressed the relation between dietary calcium intake and blood lead. In previous studies our group documented an inverse association between high milk intake and blood lead levels among delivering[16] and lactating women.[24] Similar results have been reported for other populations.[17] However, to our knowledge there are no other human trials that would allow a direct comparison of our results.

Calcium supplementation might influence blood lead levels in two ways: by decreasing absorption of ingested lead or by decreasing bone resorption. Animal and human studies support the hypothesis that calcium inhibits dietary-lead absorption at the level of the gastrointestinal tract.[11–14,25] Heard *et al.*[26] documented that in fasting adults lead uptake decreased as dietary calcium increased. Similar findings were reported by Ziegler *et al.*[27] among children studied in controlled metabolic conditions. In contrast, there are no reported studies that have evaluated this issue in relation to endogenous lead sources.

Several studies have shown that calcium requirements during lactation are met in part by increasing bone resorption.[10,28–32] Lactation is associated with substantial bone loss, estimated at 5% to 7% in the spine or hip.[28–32] Results from clinical studies that have evaluated the effects of calcium supplements on bone density across lactation have reported varied results. Some studies have shown that increasing dietary calcium intake (either by targeted nutritional counseling or by the use of supplements) may decrease bone loss during lactation.[10,31,33] In the study by Kalkwarf *et al.*,[10] calcium supplementation resulted in less bone loss among postpartum women; however, the effect was not more apparent for lactating women, and the small sample size of this group (N = 87) may have limited the statistical power of the study. In another randomized clinical trial, Cross *et al.*[31] reported small gains in bone density at the ultra distal radius and lower bone-specific alkaline phosphatase (a marker of bone resorption) among women in the calcium supplementation group (N = 7). Finally, a randomized trial (N = 30 per group)[33] conducted among African women with low calcium intake reported no differences among various markers of bone activity ex-

cept for alkaline phosphatase. Women in the calcium group had substantially lower levels of this biomarker.

A recent report that evaluated blood lead changes in 22 women during pregnancy and lactation by a high-precision lead isotopic method reported that the two women who took dietary calcium supplements had the lowest mobilization of lead from bone to blood.[34] However, the small sample precluded any conclusion regarding the protective effect of calcium supplements in relation to bone lead mobilization.

Our observation that the effect of the supplement was more apparent among women with high bone lead supports the hypothesis that some of the observed decrease in blood lead levels over the lactation period may be the result of a decrease in lead released from bone to circulation. The interaction we observed between calcium and patella lead, as opposed to tibia lead, probably reflects the higher effect of lactation-associated bone loss on trabecular bone as opposed to cortical bone.[35] In a previous report of this study in which we analyzed the contribution of bone lead to blood lead levels, we found that patella lead was a stronger determinant than tibia lead on blood lead levels in lactating women.[9,24] The sample size of our study was insufficient to completely disentangle the complex interaction of bone lead and the supplement effect.

Our study has other limitations that need consideration. First, we did not collect information regarding bone density changes or bone-remodeling biomarkers, without which we cannot directly validate our hypothesis that the supplement decreased bone resorption and, therefore, bone lead mobilization. We cannot exclude the hypothesis that the observed decrease in blood lead could reflect decreased absorption of lead at the gastrointestinal track conditioned also by the use of calcium supplements. Second, we have no direct evidence other than the pill count that participants took the supplement. The supplement was given to participants to be taken in the morning, and recent studies have shown that calcium supplements taken during the night may have higher impact.[36] This may have resulted in an underestimate of the potential impact of the supplement. Third, during the study course we had to update the radioactive source of the x-ray fluorescence instrument; this change conditioned time-dependent errors in bone lead measurements. Because only a few individuals were evaluated at baseline and at the end of the trial using a comparable source, the inferences related to changes in bone lead over time should be interpreted with caution.

In conclusion, this randomized trial demonstrates that calcium supplements may be effective in decreasing blood lead levels among women who lactate for 6 or more months. Because dietary lead absorption and bone lead mobilization are likely to be similar during pregnancy and lactation, calcium supplementation is also likely to decrease lead exposure to the fetus. These kinds of interventions are not a substitute for public health efforts to reduce environmental lead exposure from all sources; however, they may constitute an important secondary prevention effort, because dietary lead exposure is difficult to eradicate and lead exposure from long-lived bone stores is likely to persist for decades.

Acknowledgments

We would like to acknowledge the research assistance of Eugenia Fishbein, Gail Fleischschaker, PhD, Mr. Jesus Lozano, Dr. Gustavo Olais and Dr. Francisco Cabral from the Instituto Nacional de Perinatología; Dr. Dolores Saavedra, and the late Dr. Carlos Ricalde, both from the Manuel GEA Gonzalez Hospital; and also the late Dr. Rodolfo Muñoz from the Hospital de Ginecología y Obstetricia No. 4 Luis Castelazo Ayala, Mexican Social Security Institute.

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