Randomized trial of calcium glycerophosphate–supplemented infant formula to prevent lead absorption[1–3]

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ABSTRACT

Background: Although additional dietary calcium is recommended frequently to reduce the risk of lead poisoning, its role in preventing lead absorption has not been evaluated clinically.

Objective: The objective was to determine the safety and to estimate the size of the effect of calcium- and phosphorus-supplemented infant formula in preventing lead absorption.

Design: One hundred three infants aged 3.5–6 mo were randomly assigned to receive iron-fortified infant formula (465 mg Ca and 317 mg P/L) or the same formula with added calcium glycerophosphate (1800 mg Ca and 1390 mg P/L) for 9 mo.

Results: There was no significant difference between groups in the mean ratio of urinary calcium to creatinine, serum calcium and phosphorus, or change in iron status (serum ferritin, total iron binding capacity). At month 4, the median (±SD) increase from baseline in blood lead concentration for the supplemented group was 57% of the increase for the control group (0.04 ± 0.09 compared with 0.07 ± 0.10 μmol/L; $P = 0.039$). This effect was attenuated during the latter half of the trial, with an overall median increase in blood lead concentration from baseline to month 9 of 0.12 ± 0.13 μmol/L for the control group and 0.10 ± 0.18 μmol/L for the supplemented group ($P = 0.284$).

Conclusions: Supplementation did not have a measurable effect on urinary calcium excretion, calcium homeostasis, or iron status. The significant effect on blood lead concentrations during the first 4 mo was in the direction expected; however, because this was not sustained throughout the 9-mo period we cannot conclude that the calcium glycerophosphate supplement prevented lead absorption in this population. *Am J Clin Nutr* 1999;69:1224–30.

KEY WORDS Lead, calcium, phosphorus, iron, lead absorption, calcium supplementation, infant formula, infants, Massachusetts

INTRODUCTION

The current Centers for Disease Control and Prevention (CDC) blood lead threshold for concern of 0.48 μmol/L (10 μg/dL) identifies a group of lead-exposed children with blood lead concentrations in the range of 0.48–0.92 μmol/L (10–19 μg/dL). Little is known about the natural course of the exposure or the efficacy of preventive strategies. In their most recent statement on lead-poisoning prevention in children, the CDC recommends nutritional interventions that include additional dietary calcium for children with blood lead concentration in this range (1). Although evidence from animal studies indicates that calcium and phosphorus inhibit the absorption of lead from the gastrointestinal tract (2–5), there are no published reports evaluating the role of these dietary factors in the prevention of lead exposure in children (6). Because children with an elevated blood lead concentration in the range of 0.48–0.92 μmol/L constitute most of the lead-exposed children in the United States, controlled trials of nutritional interventions are needed to determine what effect, if any, nutritional supplementation has on blood lead concentrations in exposed populations.

The purpose of this pilot study was to determine the safety and acceptability of infant formula supplemented with calcium glycerophosphate in an infant population considered to be at high risk of lead exposure and to estimate the effect of this supplementation on blood lead concentrations.

SUBJECTS AND METHODS

Sample selection

The study was conducted in Lawrence, MA. Lawrence is a manufacturing city with a population of 60 000. Forty-two percent of the population is Latino (predominantly Puerto Rican and Dominican). Most of the residents are of low income and many receive public assistance. Most of the referrals for the study were made by the study's outreach worker, who recruited mothers and children from the Lawrence Women, Infants and Children Program. Additional referrals were made by health care providers and state-funded assistance programs (the Merrimack Lead Prevention

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Program and the local public assistance office). The trial began in October 1991 and subjects were enrolled continually until June 1993. Data collection was completed by April 1994.

All mothers who were interested in having their infants participate were screened in an interview to determine whether their infant met the following primary entry criteria: *1*) the parent and infant resided in a geographic area identified as high risk of lead exposure; *2*) the infant was drinking ≥600 mL (20 oz) cow milk–based formula/d; *3*) the infant was between 2.5 and 5 mo of age; *4*) the infant's birth weight was >2.0 kg; *5*) the infant's length and weight were above the 5th percentile for age; *6*) there was no history of kidney stones in first-degree relatives; *7*) the infant had no history of rickets, parathyroid dysfunction, or a recent fracture; *8*) the infant was not taking supplemental multivitamins or, if he or she was, the parent was willing to discontinue them; and *9*) the infant was not chronically taking medications other than antihistamine decongestants, fluoride supplements, or prophylactic antibiotics to prevent recurrent otitis media.

If the primary entry criteria were satisfied, the infant was enrolled in a 1-mo run-in phase to evaluate secondary entry criteria. These criteria included the following: *1*) the parent and infant returned for the 2-wk appointment; *2*) the infant tolerated a high-calcium formula for ≥2 wk; *3*) the parent, family members, and pediatrician agreed to the infant's participation; *4*) the infant's blood lead concentration was <1.21 μmol/L (25 μg/dL); *5*) the infant had no hematuria (>5 red blood cells per high-powered field); and *6*) no hypercalciuria [urinary calcium:creatinine ≥2.17, in μmol/μmol (0.77, in mg/mg)] after drinking the high-calcium formula for 2 wk.

The project manager evaluated the eligibility of the subjects and assessed the likelihood of the subjects' compliance with the study protocol. A pediatrician reviewed the clinical results from baseline measurements and determined whether they were within acceptable limits. Both the project manager and the pediatrician were blind to treatment assignment. The study was approved by the Committee for the Protection of Human Subjects at Dartmouth-Hitchcock Medical Center.

Treatment intervention and masking

Treatment was assigned at the individual level. Infants who successfully completed the run-in phase and satisfied all of the entry criteria were randomly assigned to 1 of 2 groups. Infants in the control group received standard, iron-fortified infant formula (Enfamil with iron; Mead Johnson Co, Evansville, IN) containing 465 mg Ca and 317 mg P/L. The supplemented group received the same formula supplemented with calcium glycerophosphate to a concentration of 1800 mg Ca and 1390 mg P/L. Formula was dispensed monthly for 9 mo. Parents were asked to give only the study formula to their infants and not to introduce cow milk until after completion of the study. Exceptions to this rule were made if the mothers reported that their infants were drinking >1440 mL (48 oz) infant formula/d. In these cases, the mothers were asked to limit the intake of study formula to 1440 mL/d and to use standard infant formula for any additional feedings.

The standard infant formula and the calcium glycerophosphate–supplemented formula were both supplied in 453-g (1-lb) cans of powder with identical labeling. Each can made ≈3600 mL (120 oz) infant formula, which was prepared with 1 scoop (provided in the can) of powder for every 60 mL water. There was no discernible difference in taste or color between the 2 formulas.

All on-site study personnel and subjects' parents were blind to treatment assignment. An off-site employee who was not blind to treatment assignment was responsible for distributing the formula. Before the study began, the data manager used the random number generator function (RAND) of Microsoft Excel (Microsoft Inc, Redmond, WA) to assign a treatment group to each identification number. Treatment assignments were given to the formula distribution manager, who then prepared 12 cans of formula per month for every identification number according to the randomization scheme. Subjects eligible to be randomly assigned to treatment groups were assigned consecutive identification numbers and another unique identifier by the project manager. The subjects received the formula corresponding to their identification number on a monthly basis. On-site personnel recorded the number of cans distributed to each subject and this information was matched to the number of cans dispensed for each identification number that was recorded on logs kept by the formula distribution manager.

As far as we know, study personnel and participants were not aware of treatment assignment. However, no assessment was made to determine whether the employees or participants correctly guessed their treatment assignment.

Collection and analysis of samples

One month after randomization, parents were asked to return with their infants every month for the next 9 mo. Mothers were interviewed at each visit to assess their infant's tolerance of the formula, identify side effects or complaints, and monitor the infant's average daily formula intake. The infants' lengths and weights were measured monthly. Urine samples were obtained at baseline and months 1, 2, 4, 6, and 9 to monitor the excretion of calcium and creatinine. Blood samples were obtained at baseline and months 4 and 9 to measure concentrations of blood lead, serum calcium, serum phosphorus, and serum ferritin; total iron binding capacity (TIBC); erythrocyte protoporphyrin; and hematocrit. Environmental samples of household dust and water were collected ≈1 mo after randomization. Environmental sampling was repeated if participants moved to a new residence.

All blood samples were collected by drawing ≈2 mL venous blood from the antecubital area through a butterfly and syringe and transferred to a K_3-EDTA–containing Vacutainer tube (Becton Dickinson and Co, Orangeburg, NY). The samples were analyzed for extractable lead by the Kettering Laboratory, University of Cincinnati, which procured all blood sampling materials. To preclude lead contamination of these materials, randomly selected samples from each manufacturer's lot of blood specimen containers, syringes, and butterfly needles were washed with acid and analyzed for lead before shipment to the clinical site. Blood lead was analyzed by using anodic stripping voltametry. The procedure described is a modification of the method in the Environmental Science Associates (Boston) instruction manual for the 3010A Trace Metal Analyzer (7). Laboratory personnel were blind to treatment assignment.

The Kettering Laboratory is 1 of 6 reference laboratories used by the proficiency testing program run by the CDC, Health Resources and Services Administration, and the University of Wisconsin. This laboratory also participates in several other proficiency programs for blood lead analysis, including those run by the New York State Department of Health and the College of American Pathologists (Skokie, IL). In addition to these programs, human blood samples—whose lead concentra-

tions were determined previously by isotope dilution mass spectrometry—were incorporated into the analysis of the samples for this study. In these instances, the blood samples that had been analyzed by isotope dilution mass spectrometry were given a fictitious name and analyzed blind. There were 2 such samples analyzed per 60 unknowns. Finally, we periodically sent duplicate samples of blood collected from study personnel, which were labeled as study specimens. Control charts documenting performance over extended intervals were maintained for these samples to determine whether any persistent displacement of results occurred. The minimum detection limit of the blood lead method used is 0.06 μmol/L (1.3 μg/dL) and the SD of the error for repeated measurements (precision) is 0.04 μmol/L (0.8 μg/dL) when the concentration of lead in the specimen is ≤0.48 μmol/L (10 μg/dL). Precision falls to an SD of 0.09 μmol/L (1.8 μg/dL) at a blood lead concentration of 2.22 μmol/L (46 μg/dL).

To measure environmental lead exposure, dust samples were collected by placing a rubber-backed, olefin-fiber mat in the doorway of the participant's home and asking family members not to clean or shake the mat. After 30 d, a dust sample was collected from a 25 × 25 cm area on the mat by using an air-monitoring vacuum pump. The dust samples were digested by using 1 mol HNO_3/L and this solution was filtered and analyzed for lead content by atomic absorption spectroscopy. Water samples, collected from the kitchen tap during the home visit, were acidified by adding 0.5% concentrated HNO_3 by volume and analyzed for lead by atomic absorption spectroscopy.

Children were weighed while wearing a dry diaper and light clothing on a baby scale that was calibrated to the nearest 20 g. Accuracy of the scale was verified and monitored annually by the Massachusetts Department of Weights and Measures. Recumbent lengths were measured with a standard length board that was marked to the nearest 0.3 cm. All children were measured in a supine position without shoes or hair accessories. Study personnel were trained to take anthropometric measurements before the start of the study and the accuracy of their measurements was monitored regularly during the trial.

Laboratory norms

Decisions regarding abnormal results were made independent of treatment assignment according to the following established normal values in infants: serum calcium, 2.13–2.72 mmol/L (8.55–10.91 mg/dL); serum phosphorus [age <6 mo: 1.80– 2.71 mmol/L (5.58–8.38 mg/dL); 6–12 mo: 1.61–2.51 mmol/L (4.98–7.78 mg/dL); and 12–18 mo: 1.56–2.31 mmol/L (4.82 –7.14 mg/dL)]; blood in urine, ≤5 red blood cells per high-powered field; urinary calcium:creatinine in μmol/μmol (mg/mg) [baseline value: <2.26 (0.8); age 100–199 d: <2.54 (0.9); age 200–299 d: <1.69 (0.6); and age 300–730 d: <1.41 (0.5)]; and serum ferritin >12 μg/L (8).

Statistical analysis

Data were entered without awareness of intervention allocation. The data analyst reviewed data regularly for accuracy and completeness, unblinded to treatment assignment. Analyses were based on the original treatment assignment and all follow-up measurements were included. The predefined primary outcome measure for this trial was the blood lead concentration of the control and supplemented groups.

Wilcoxon rank-sum tests were used to test for differences in group medians for all variables that were not normally distributed (blood lead concentration, change in blood lead concentration, urinary calcium:creatinine, serum ferritin, TIBC, and child's age). Unpaired *t* tests were used to test for differences between group means of normally distributed data (serum calcium, serum phosphorus, formula intake, calcium intake, maternal age and education, and infant length and weight). STATA statistical software (release 5.0; STATA Corporation, College Station, TX) was used for the analyses.

RESULTS

Participant flow

Five hundred sixteen mothers were interviewed to determine whether their children were eligible to enroll in the 1-mo run-in phase. Seventeen percent (n = 85) of these infants were excluded from the study because they did not meet the primary entry criteria. The most common reason for exclusion at this stage was a family history of kidney stones (n = 73; 86%). Other reasons for exclusion included the following: chronic use of medications or vitamins (n = 5), a birth weight <2 kg (n = 3), a history of bone disease or a high serum calcium concentration (n = 1), and failure to meet other requirements pertaining to the infant's residence, age, or formula tolerance (n = 5). Twenty-three percent (n = 117) of the mothers decided not to enroll their infants after hearing all the details of the study. The remaining infants (n = 314; 61%) were enrolled in the run-in phase.

One-third (n = 103) of the infants who participated in the run-in phase completed it successfully and were randomly assigned to the control or supplemented group. Three quarters (n = 162; 77%) of the infants who participated in the run-in phase failed to return for the second appointment. We attempted to contact all parents who missed scheduled appointments, but in most instances we were unable to determine the reason for noncompliance. The project manager decided not to randomly assign 27 (12.8%) infants because she thought they were unlikely to participate in the trial for the full 9 mo months. Only 3 children (1%) were excluded on the basis of the physiologic criteria evaluated during the run-in phase. Median baseline blood lead concentrations for those infants who did not complete the run-in phase were not significantly different from the concentrations of those who were randomly assigned into the study.

Complete laboratory data were obtained for 81 (78.6%) of the 103 infants who were randomly assigned into the study. Seventy-five infants consumed the study formula for the entire 9-mo study period. Six infants completed the study 1–3 mo early, 4 of whom were in the supplemented group. For these infants, month 9 measurements refer to the measurements made at their last visit. Twenty-one percent (n = 22) of the children who were randomly assigned into the study dropped out before completion. Final blood and urine samples were obtained from 10 of the 23 dropouts. Drop out rates were equivalent for both groups (11 control and 11 supplemented subjects). Reasons for leaving the study after randomization included moving from the Lawrence area (4 control and 3 supplemented subjects), switching to cow milk or another formula (4 control subjects and 1 supplemented subject), prolonged diarrhea that the mother attributed to the milk (1 supplemented subject), and solidification of the powder when the formula was prepared in the microwave (1 supplemented subject). We were unable to determine why 8 infants (3 control and 5 supplemented subjects)

TABLE 1
Demographic, physiologic, and environmental characteristics at baseline[1]

	Control group (n = 52)	Supplemented group (n = 51)
Mother's marital status [n (%)]		
Single	26 (50.0)	22 (44.0)
Married	12 (23.1)	17 (34.0)
Divorced, separated, or widowed	14 (26.9)	11 (22.0)
Race [n (%)]		
White	3 (5.8)	2 (3.9)
Latino	48 (92.3)	47 (92.2)
Other	1 (1.9)	2 (3.9)
Maternal age (y)[2]	25.2 ± 5.7	25.5 ± 5.8
Maternal education (y)[2]	11.0 ± 2.4	11.2 ± 2.9
Household per capita income ($)[3]	2545 ± 1359	2772 ± 1952
Child's age (mo)[3]	3.1 ± 1.4	3.2 ± 1.3
Length (cm)[2]	67.8 ± 3.7	63.6 ± 3.3
Weight (kg)[2]	6.7 ± 1.2	6.9 ± 1.2
Urinary calcium:creatinine (μmol/μmol)[3,4]	0.3 ± 0.6	0.3 ± 0.6
Serum calcium (mmol/L)[2]	2.5 ± 0.1	2.6 ± 0.1
Serum phosphorus (mmol/L)[2]	2.0 ± 0.2	2.0 ± 0.2
Serum ferritin (μg/L)[3]	69.9 ± 55.9	45.1 ± 41.6[5]
TIBC (μmol/L)[3]	50.0 ± 9.6	53.5 ± 5.6
Dust lead (μg/g)[3]	454.3 ± 850.9	601.3 ± 635.8
Water lead (μg/L)[3]	1.2 ± 6.6	1.0 ± 2.5

[1] TIBC, total-iron-binding capacity.
[2] $\bar{x}$ ± SD.
[3] Median ± SD.
[4] Measured in micromoles. To convert to mg/mg, divide by 2.8224.
[5] Significantly different from control group, P = 0.025.

dropped out because repeated attempts to contact the parents were unsuccessful.

The 22 dropouts did not differ significantly from those who completed the study in any of the following measures: mother's age, mother's education, infant's age, infant's baseline length or weight, baseline laboratory values (blood lead, urinary calcium:creatinine, serum calcium, serum phosphorus, serum ferritin, and TIBC), and environmental dust or water lead concentrations. Mean serum phosphorus at baseline was slightly higher in the dropouts than in the subjects who completed the study (6.5 compared with 6.2 mmol/L, respectively) and serum ferritin was lower among the dropouts (43.0 compared with 57.2 μg/L), but neither was significantly different between the groups (P = 0.06 and P = 0.18, respectively).

The control and supplemented groups did not differ significantly at baseline in demographic, physiologic, or environmental characteristics (**Table 1**), except for the median serum ferritin concentration, which was significantly higher in the control group. The mean age of all infants was 5.1 mo at baseline, 9.3 mo at month 4, and 14.0 mo at month 9.

Outcome measures

On the basis of parents' reports, there were no significant differences in mean intakes of infant formula at baseline, month 4, and month 9 between the control and supplemented groups (**Table 2**). Infants maintained a mean (±SD) intake of ≥720 ± 12.4 mL/d (24 oz/d) until the end of the study, even though they were well into their second year of life. The mean calcium intake for infants receiving the supplemented formula was >4 times that of children in control group at months 4 and 9. There were no significant differences in median urinary calcium:creatinine values between groups.

There was no significant difference in mean change in length and weight from baseline to month 9 between the supplemented and control groups (data not shown). We also compared growth velocity between 6 and 12 mo of age and found no significant difference between the 2 groups.

Median blood lead concentrations were not significantly different between the 2 groups at baseline, month 4, or month 9, but the median change in blood lead concentrations between baseline and month 4 was significantly lower in the infants receiving the calcium glycerophosphate–supplemented formula than in the control group (**Table 3**). During the first half of the study, the median blood lead concentration for the control group increased by 83% (from 0.12 to 0.22 μmol/L) compared with an increase of 42% for the supplemented group (from 0.12 to 0.17 μmol/L). However, this effect did not persist during the latter half of the study; the median change in blood lead between baseline and month 9 was not significantly different between the 2 groups.

The analysis of change in blood lead concentration was repeated, excluding children who were taking iron supplements (*see* below) and the findings did not change; the median change from baseline to month 9 was the same for control subjects (0.12 μmol/L) and slightly lower for the supplemented group (0.07 μmol/L) (P = 0.08). This analysis was also repeated, excluding the infants who did not drink the study formula for the full 9-mo study period; again, the results did not change.

The blood lead concentration of one child in the supplemented group rose to 1.06 μmol/L (22 μg/dL) by month 9. This infant

TABLE 2
Formula and calcium intakes and calcium excretion by group[1]

	Control group	Supplemented group
Formula intake (mL/d)		
Baseline	1029 ± 186 [52]	966 ± 252 [51]
Month 4	864 ± 279 [46]	948 ± 255 [43]
Month 9	738 ± 372 [35]	867 ± 390 [27]
Calcium intake (mg/d)		
Baseline	477.8 ± 86.6 [52]	1740.7 ± 451.4[2] [51]
Month 4	402.1 ± 130.1 [46]	1707.3 ± 456.6[2] [43]
Month 9	343.6 ± 173.1 [35]	1563.0 ± 703.6[2] [27]
Urinary calcium:creatinine (μmol/μmol)[3]		
Baseline	0.40 ± 0.56 [49]	0.37 ± 0.59 [51]
Month 4	0.42 ± 0.34 [38]	0.28 ± 0.56 [42]
Month 9	0.34 ± 0.45 [39]	0.28 ± 0.54 [38]

[1] $\bar{x}$ ± SD, except for urinary calcium:creatinine values (median ± SD); *n* in brackets.
[2] Significantly different from control group, $P < 0.001$.
[3] To convert to mg/mg, divide by 2.8224.

had no rise in blood lead concentration between baseline and month 4, at which time his formula intake was high; however, he was drinking only 150–240 mL (5–8 oz) supplemented formula per day during the last 3 mo of the study.

Adverse events

Ten children, who were evenly distributed between the control and supplemented groups, had at least one urine sample with a ratio of urinary calcium to creatinine above the age-related norm. None of these urine samples indicated hematuria. On repeat sampling, 2 children continued to have a high ratio of urinary calcium to creatinine: 1 in the control group and 1 in the supplemented group. The infant in the supplemented group was switched to an infant formula containing a reduced calcium and phosphorus content (1200 mg Ca and 920 mg P/L) and consumed this formula for the duration of the study.

One child from the control group had a serum calcium concentration that was elevated slightly above the norm of 2.72 mmol/L at month 4. By month 9, this infant's serum calcium concentration was within the normal range. Many subjects (n = 22) had serum phosphorus concentrations that were lower than age-related norms. In only one case was the concentration <1.3 mmol/L (4 mg/dL). The proportion of children who had abnormally low serum phosphorus concentrations at months 4 and 9 did not differ significantly by group. These children were monitored according to study protocol and no changes were made in their treatment. None of the infants in the study had an abnormally high serum phosphorous concentration.

Thirteen children were given nightly iron supplements during the study because they had low serum ferritin concentrations. Five of these children were in the control group and 8 were in the supplemented group. Half of these children (n = 7) also had low serum ferritin concentrations at the end of the study; these children were equally distributed between control and supplemented groups. Three additional children in the supplemented group had low serum ferritin concentrations at month 9. Because these children were no longer participating in the study when the results were received, their pediatrician was contacted for follow-up. A detailed summary of iron status in this population was published elsewhere (9).

DISCUSSION

Calcium and phosphorus are the most thoroughly studied nutritional factors in lead metabolism (6). Animal studies indicate that both calcium and phosphorus inhibit absorption of lead from the gastrointestinal tract (2, 4), that calcium deficiency results in increased lead absorption (10) that calcium supplementation above normal dietary requirements results in decreased lead absorption (5), and that the inhibitory effect of calcium is short-lived, as one would expect if calcium were competing with lead for transport across the intestinal epithelium. (5). In one study (3), both lead and calcium were shown to bind to 2 mucosal proteins in the small intestine of rats and binding of lead was markedly diminished by co-administration of calcium. This confirms that calcium inhibits the absorption of lead in some mammals by binding it to and displacing it from a common mucosal carrier in the lumen of the small intestine.

Results of epidemiologic studies are also consistent with the principle that dietary calcium inhibits lead absorption in children (11) and adult women (12). It has also been suggested that lower calcium intakes in African American children may explain the higher blood lead concentrations in this racial group than in the rest of the population (11, 12). Finally, small studies of absorption of radiolabeled lead in human adults confirm the findings of animal studies for both calcium and phosphorus (13, 14). To our knowledge, the present study was the first attempt to supplement the formula of term infants with large amounts of calcium and phosphorus. This study showed that calcium glycerophosphate supplementation is acceptable to infants. Supplementation did not affect urinary calcium excretion and, therefore, probably does not increase the likelihood of nephrolithiasis. This finding supports earlier studies by us (8) and others (6, 15) that showed that increases in the calcium content of the formula are not associated with increased urinary calcium excretion in infants, as long as the ratio of calcium to phosphorus in the infant formula does not change. In addition, supplementation with calcium glycerophosphate during a period when infants are at high risk of developing iron deficiency did not result in higher rates of iron deficiency. The well-documented inhibitory effect of calcium on iron absorption (16–22) probably has little clinical significance in growing infants consuming iron-fortified infant formula.

The generalizability of these findings is limited by the high rate of attrition and by our lack of data on calcium absorption. This precludes our ability to speculate on whether Latinos absorb calcium differently than other racial or ethnic groups. Finally, calcium glycerophosphate is not a commonly used salt

TABLE 3
Blood lead concentrations[1]

	Control group	Supplemented group
Blood lead (μmol/L)[2]		
Baseline	0.12 ± 0.06 [52]	0.12 ± 0.07 [51]
Month 4	0.22 ± 0.11 [45]	0.17 ± 0.08 [44]
Month 9	0.24 ± 0.13 [41]	0.23 ± 0.17 [40]
Change in blood lead (μmol/L)		
4 mo−baseline	0.07 ± 0.10 [45]	0.04 ± 0.09[3] [44]
9 mo−baseline	0.12 ± 0.13 [41]	0.10 ± 0.18 [40]

[1] Median ± SD; *n* in brackets.
[2] To convert to μg/dL, divide by 0.04826.
[3] Significantly different from control group, $P = 0.039$.

for calcium supplementation. It was chosen over other calcium salts because of its high solubility in the formula, but we do not know how well calcium is absorbed from it relative to other salts.

Our results support an inhibitory effect of calcium and phosphorus supplementation on blood lead in infants. During the first 4 mo of the study, children consuming the high-calcium formula had a median increase in blood lead that was 57% of the median increase observed in the control group, indicating that a treatment effect was present before 12 mo of age. However, this effect was attenuated by the end of the study because, during the latter half, the calcium-supplemented group experienced a median rise in blood lead concentration that was slightly greater than that in control subjects. The result is that there was no difference by group in the overall increase in blood lead concentration. Because most children in this study experienced a rise in blood lead concentrations that was much less than we anticipated, we were unable to estimate the effect of formula supplementation on clinically significant lead exposure in young children.

Why was the calcium-phosphorus effect observed during the first 5 mo and not sustained during the latter half of the study? First, the significant difference in the rise during the first period could have been due to chance. If the blood lead measurements in the supplemented group at baseline were systematically biased upward with respect to their true value, then regression to the mean would explain the results for the second portion of the study. This explanation is supported by a significant negative correlation ($r = -0.44$, $P < 0.01$) between baseline blood lead concentrations and changes in blood lead concentrations (4 mo − baseline) for the supplemented group but not for the control group. Alternatively, the negative correlation for the supplemented group could indicate that the supplement was most effective for those children who had higher lead concentrations at baseline.

Second, it is possible that the decreased frequency of feedings accounted for the attenuation of the effect during the latter half of the study. Although mothers' reports indicated that their infants maintained their consumption of infant formula throughout the second half of the study, it is plausible that the frequency of formula ingestion decreases as children grow older and they are able to consume a greater volume of formula at each feeding. It is also possible that despite reportedly high intakes of formula during the second half of the study, there could have been widespread noncompliance during this period. If infants systematically discontinued consuming the study formula at 12 mo of age, any treatment effect seen in the first half of the study would not be observed in the second half of the study. Because frequent administration of the infant formula is necessary for competitive inhibition of lead absorption, either a reduction in the frequency of feedings or noncompliance among study participants could explain the diminished effect of the calcium supplement. Either of these conditions, coupled with increased exposure to lead during the second year of life, would also explain why similar numbers of children in each group (4 per group) experienced clinically significant increases in blood lead concentrations of >0.48 μmol/L during the second half of the study.

Median lead concentrations observed in this study population are comparable with those seen in other populations currently considered to be at high risk (23), in which >20% of 1–2-y-old children had blood lead concentrations ≥0.48 μmol/L (10 μg/dL). However, only one child in our study population (1.2%) had a blood lead concentration >0.48 μmol/L; this is because the distribution of blood lead among our study participants was much less skewed. In addition, the lead exposure was less than we expected based on earlier data collected from a cohort study in Cincinnati (24). This phenomenon may reflect the trend over time that has produced large declines in blood lead concentrations in the entire US population and is probably due to the systematic elimination of lead from gasoline and from food in this country (25, 26). Moreover, Massachusetts has a strict house abatement law, coupled with a system of enforcement, that has resulted in the abatement of lead in >4000 houses in Lawrence; this could have reduced further the lead exposure in children who live there. The results of the water analyses suggest that tap water was not a significant contributor of lead in this population. Dust samples collected from the door mats indicated that lead was present in the home environments of these infants. However, because there have been no studies correlating dust lead collected in this manner with blood lead concentrations in children, we were unable to determine the degree of risk of environmental lead exposure for these children.

Selection bias could have also contributed to the lower level of exposure among study participants. The 1-mo run-in phase was designed to select mothers and infants who were most likely to comply with the study protocol and minimize dropout after randomization. However, by doing this we may have selected infants who were least likely to be exposed to lead. To investigate this possibility, we attempted to obtained follow-up blood lead concentrations for all children who participated in the run-in phase but who were not randomly assigned into the study. These children were screened subsequently at the Massachusetts Lead Prevention Program or by their pediatrician. We were able to obtain the results of lead tests for 103 of the 202 infants who participated in the run-in phase only. These children had a mean blood lead concentration of 0.30 μmol/L (6.2 μg/dL), which was not significantly different from the mean blood lead concentration of 0.27 μmol/L (5.5 μg/dL) in the children who completed this study. Nonetheless, it is possible that the children for whom we were unable to obtain follow-up measures had higher blood lead concentrations. Mothers who have their children screened for lead probably are more likely to be concerned about the risks associated with lead poisoning and have a greater awareness of risk reduction measures. We caution investigators to be aware of the possible bias from sample selection in planning prospective studies of lead exposure.

This study was limited by its small sample size, which was not adequate to test for significant differences in the change in blood lead concentrations observed in this study population. The sample size for this pilot study was chosen to evaluate the safety and acceptability of the calcium supplement and to provide an estimate of its effect on lead absorption. We demonstrated the safety and acceptability of an iron-fortified infant formula supplemented with calcium glycerophosphate, but were unable to determine its effect on blood lead concentrations in children who have clinically significant environmental exposure to lead. Additional studies are required to test the hypothesis. We believe a follow-up study is most likely to show an effect in a population that has a higher lead exposure than the children in this study.

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