Contribution of Lead from Calcium Supplements to Blood Lead

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We conducted a case–control study to determine the contribution of lead to blood from consumption of calcium supplements approximating the recommended daily intakes over a 6-month period. Subjects were males and females ages 21 to 47 years (geometric mean 32 years) with a geometric mean blood lead concentration of 2.5 µg/dL. They were subdivided into three groups. One treatment group (n = 8) was administered a complex calcium supplement (carbonate/phosphate/citrate) and the other treatment group (n = 7) calcium carbonate. The control group (n = 6) received no supplement. The lead isotopic compositions of the supplements were completely different from those of the blood of the subjects, allowing us easily to estimate contribution from the supplements. The daily lead dose from the supplements at 100% compliance was about 3 µg Pb. Three blood samples were taken at 2-month intervals before treatment to provide background values, and three were taken during treatment. Subjects in the treatment group were thus their own controls. Lead isotopic compositions for the complex supplement showed minimal change during treatment compared with pretreatment. Lead isotopic compositions in blood for the calcium carbonate supplement showed increases of up to 0.5% in the $^{206}Pb/^{204}Pb$ ratio, and for all isotope ratios there was a statistically significant difference between baseline and treatment (p < 0.005). The change from baseline to treatment for the calcium carbonate supplement differed from that for both the control group and the group administered the complex supplement. Blood lead concentrations, however, showed minimal changes. Variations in blood lead levels over time did not differ significantly between groups. Our results are consistent with earlier investigations using radioactive and stable lead tracers, which showed minimal gastrointestinal absorption of lead in the presence of calcium (± phosphorus) in adults. Even though there is no discernible increase in blood lead concentration during treatment, there are significant changes in the isotopic composition of lead in blood arising from the calcium carbonate supplement, indicating a limited input of lead from diet into the blood. Because calcium carbonate is overwhelmingly the most popular calcium supplement, the changes we have observed merit further investigation. In addition, this type of study, combined with a duplicate diet, needs to be repeated for children, whose fractional absorption of lead is considerably higher than that of adults. *Key words*: blood lead, calcium supplements, isotopes, lead. *Environ Health Perspect* 109:283–288 (2001). [Online 2 March 2001]
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The increasing incidence of fractures related to osteoporosis in both females and males has led to efforts to increase bone mass, especially in young children and adolescents (*1*). It is recognized, even in developed countries such as the United States, that daily intakes of calcium among children are below recommended levels (*2,3*). One approach to increase bone mass has been with vitamin and mineral supplementation; another has been fortification of foodstuffs such as cereals.

In addition to the potential to increase bone mass, calcium supplementation offers other advantages at various stages of life. For example, calcium supplementation reduces the incidence of pregnancy-induced hypertension and preeclampsia (*4*). In addition, Koo et al. (*5*) showed that supplementation with 2 g calcium per day increased fetal bone density in subjects whose calcium intake was normally < 600 mg per day. Of relevance to our study is the recognition that lead absorption by the gastrointestinal tract is increased in calcium-deficient diets (*6*). Absorption of lead by the gastrointestinal tract is inversely related to the amount of calcium present (*6,7*). Furthermore, calcium supplements had a protective effect by significantly reducing blood lead levels in pregnant women whose diets were deficient in calcium (*8,9*). The increased mobilization of skeletal lead during pregnancy and lactation was attributed to low calcium intake (*10*).

As people have been encouraged to take calcium supplements, several investigators have drawn attention to their potential lead toxicity (*11–15*). Furthermore, the lead concentrations in calcium supplements were the subject of California legal action, Proposition 65 (*16*).

Any lead contained in calcium supplements will be introduced in conjunction with the calcium. Lead binds tightly to the same transport proteins used by calcium, but the binding affinity of lead is at least twice that of calcium (*17*). Because the same transport mechanism is operative for absorption of lead and calcium from the gastrointestinal tract, there is resulting competitive interaction between lead and calcium (*18*).

The agreement reached between the California Attorney General and supplement manufacturers for Proposition 65 was an acceptable daily intake of < 1.5 µg Pb/g Ca for adults (*15,16*). Even at these levels, however, supplements potentially contribute a significant proportion of the daily intake of lead that is now < 5 µg/day in the United States (*19*).

We were concerned about the scientific validity of the California judgment in light of earlier investigations from tracer studies in both animals and humans demonstrating limited uptake of lead from the gastrointestinal tract in the presence of calcium (± phosphorus; Figure 1) (*20–24*). These experiments, however, were undertaken at times of considerably higher intakes of lead with small numbers of subjects and mostly over limited time periods. The publicity arising from the California legal action was of concern because we had just begun a clinical trial in which subjects were given calcium supplements to verify the hypothesis that calcium supplementation minimizes the mobilization of skeletal lead during pregnancy and lactation. To satisfy ourselves and participants in our trial of the potential safety of calcium supplements and the minimal contribution to blood lead, we undertook a case–control study in which adults consumed calcium supplements containing lead whose isotopic composition was different from that in their current blood.

The question we asked was, does the amount of lead in calcium supplements increase blood lead concentration or change the isotopic composition of lead in blood at the recommended daily intake levels of calcium?

Methods

Rationale. We demonstrated previously that the lead isotopic composition of multigenerational Australians was different from that of subjects from most other countries (*10*). We used this concept, first suggested by Manton (*25*), in our longitudinal study of

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mobilization of lead from the maternal skeleton during pregnancy and lactation. Commercially available Australian calcium supplements, which are typically made from overseas components, were administered to the subjects.

Subjects and dosing. Subjects, 8 males and 13 females, ranged in age from 21 to 47 years with a geometric mean of 32 years. We attempted to assign subjects randomly to one of three groups, two treatment and one control group, but two subjects expressed a desire to be in the control group. This does not, however, confound our results, because the treatment subjects formed their own controls by having three baseline measurements. The others were randomly assigned to the treatment groups. Ethics clearance was obtained from Macquarie University.

Sampling. A trained phlebotomist obtained 2–3 mL of venous blood from each subject. Three samples were collected at quarterly intervals to establish a background value before treatment. During treatment, samples were collected bimonthly for 6 months of calcium supplementation. Thus, each subject in the treatment group acted as his or her own control. Because of changes observed by the group given the calcium carbonate supplement, an additional blood sample for this group was taken 2 months after the treatment ended to see if there was a trend toward the baseline values.

Treatment. We originally intended to use a popular calcium carbonate product, but it was not possible to obtain the required quantity of a single manufacturing batch from the supplier. However, for the two treatment supplements, sufficient product was obtained from the one batch of production to minimize variability in the source of calcium and hence lead. The possibility of supplying the control group with a placebo was investigated but discarded because of production difficulties for such a small batch. The product details are listed in Table 1. The complex supplement was to be taken 3 times daily, with meals and at bedtime, because the fasting state is a time when most parathyroid hormone-mediated bone resorption occurs (*26*). The calcium carbonate was taken twice daily after meals.

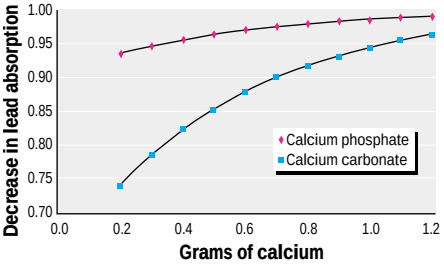

Figure 1. Decreases in lead absorption in the presence of calcium and phosphorus [data from Blake and Mann (*20*)].

Two subjects experienced nauseous reactions which they possibly attributed to the calcium supplements. One subject, given the complex product, had just conceived and was therefore moved to the control group. The other experienced nausea taking the calcium carbonate product; the subject was switched to the alternative supplement and thereafter showed no adverse effects.

In this pilot investigation, we did not attempt to undertake a dietary study apart from a questionnaire. However, our previous and ongoing pregnancy research showed that the daily calcium intake of migrant and Australian subjects is low (about 500 mg Ca/day) compared with the recommended levels for subjects participating in U.S. studies on pregnancy and lactation (*27–32*). In these studies, based on diaries and recall, calcium intake was estimated at 1,200–1,800 mg Ca/day, compared with 300–800 mg Ca/day for our subjects (*10*).

Questionnaire. On one occasion we gave the participants a questionnaire that placed a special emphasis on the intake of potentially calcium-bearing foods.

Analyses. All sample preparation was performed in purpose-built low-contamination laboratories (clean rooms) incorporating features such as filtered air intake and laminar flow hoods. To minimize sample heterogeneity, the total blood sample was predigested in ultrapure concentrated nitric acid and an aliquot of < 1g removed to a clean Teflon vessel. A ^{202}Pb spike solution of known isotopic composition and lead concentration (~ 10 ng/g) was added to the aliquot to obtain the concentration of lead and isotopic composition of the unknown sample in the one analysis (this is known as the isotope dilution method). (^{202}Pb is not naturally occurring and is produced in cyclotrons as a by-product of preparation of thallium used in treatment of thyroid abnormalities; it has a half-life of about 3×10^5 years.) We analyzed the calcium supplements for their lead isotopic composition by first spiking with the ^{202}Pb spike and then dissolving/digesting in ultra-pure nitric acid. An aliquot was removed to a clean Teflon vessel and its pH was adjusted using clean ammonia. We separated lead from the calcium by passing the solution through a chelex filter. We further separated lead from other interfering ions using anion exchange chromatography in a bromide medium.

For isotope ratio measurement, we loaded fractions of the purified lead samples onto a rhenium filament using the silica gel technique (a mix of dilute phosphoric acid and purified silica gel) and analyzed them for lead isotope composition (and lead concentrations by isotope dilution) on a thermal ionization mass spectrometer (VG-ISOMASS 54E; VG, Winsford, Cheshire, UK) run in fully automatic mode. Isotopic ratios were measured as ^{208}Pb/^{206}Pb, ^{207}Pb/^{206}Pb, and ^{206}Pb/^{204}Pb. Precision estimates on the isotopic ratios have been defined by a repetition of the digestion/lead separation/mass spectrometry stages of the same samples of blood, urine, and water. The precisions we allocate our data are ± 0.2% (2 σ) on the ^{206}Pb/^{204}Pb ratio, and ± 0.1% on the ^{208}Pb/^{206}Pb and ^{207}Pb/^{206}Pb ratios. Data are normalized to the accepted values of the National Institute of Standards and Technology (NIST) SRM 981 by applying a correction factor of +0.08% atomic mass units to allow comparisons between laboratories. We obtained a measurement of the environmental lead acquired by the sample throughout the entire preparation analysis procedure in the form of a lead blank measurement. The amount of contamination detected in blanks was generally around 200 pg for blood and from 300 to 900 pg for the calcium supplements. Because the blanks contributed negligibly to the lead in the sample, no blank corrections to the data were performed.

Data treatment. We analyzed the three isotopic ratios (^{208}Pb/^{206}Pb, ^{207}Pb/^{206}Pb, and ^{206}Pb/^{204}Pb) and lead concentrations using a 2 (baseline and treatment) × 3 (days 1, 2, and 3 of baseline and treatment) × 3 (the control, calcium carbonate, and calcium citrate groups) factorial design. The first two factors were within-subject factors.

Results

Results are summarized in Table 2, and a comparison of means of the ^{206}Pb/^{204}Pb ratio and blood lead concentrations for each

Table 1. Product information.

Composition	Complex product: Calcium citrate	Complex product: Calcium phosphate	Complex product: Calcium amino acid	Calcium carbonate (chewable)
Weight Ca compound (mg)	300	325	200	1,250
Equivalent Ca (mg)	64	126	40	500
Daily total Ca (mg)		920		1,000
Daily dosage		3 times after meals; 1 on retiring		2 times after meals
^{206}Pb/^{204}Pb		20.14	19.70	
Pb (µg/kg)		293	700	
Daily Pb intake (µg/day)		2.8	3.2	

group is shown in Figures 2 and 3. Compliance for the 3 months of the treatment period ranged from 0 to 100% for each month. It was expected that changes in pretreatment blood samples may have been larger because sampling covered the Christmas holidays, a time of increased dietary intake and consumption of unusual foods. We sampled approximately 1 month after Christmas to minimize potential effects of abnormal dietary intakes, because the mean life of lead in blood of adults is approximately 20 days (*33*, *34*).

Blood lead concentrations. We observed a slight downward trend over time in blood lead concentrations (Table 2, Figure 3), which was reflected in a main effect of treatment ($F_{(1,14)} = 5.94$, $p = 0.029$) and also in a day main effect ($F_{(2,28)} = 4.12$, $p = 0.027$). However, the change over days did not differ for baseline and treatment (for the day-by-treatment interaction, $F_{(2,28)} = 0.34$, $p = 0.717$), and, as will be seen below, there was no suggestion of a group-by-treatment interaction.

Blood lead isotopic ratios. Apart from the anomalies described below, there were minimal changes in isotopic ratios in the control subjects and those given the complex supplement (Figure 2). In contrast, there were detectable increases in $^{206}Pb/^{204}Pb$ ratio during administration of the calcium carbonate toward the values in the supplement (Figure 2; $p = 0.002$). Significant changes in the calcium carbonate group from baseline to treatment also occurred for $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$ ($p < 0.0005$ and $p = 0.001$, respectively). The blood sample taken 2 months after treatment ended showed a decrease toward the baseline values for $^{206}Pb/^{204}Pb$ in some cases, indicating that the increases toward the supplement noted above were a valid effect. There was also a small decrease in blood lead concentration after cessation of treatment (Figure 3).

Anomalies in subjects. As is becoming increasingly evident in human studies, individual susceptibility to toxins or treatments plays a major role in determining impacts on health, as do changes in the environment of subjects. In our study, such problems arose for several subjects. For example, male subject 1305 undertook renovations using unsafe methods to an external garage with lead paint just before the start of treatment. Both he and his wife (subject 1306) experienced a sharp decrease in $^{206}Pb/^{204}Pb$ ratio in blood Pb and an increase in blood lead (PbB) concentration (Table 2). The low $^{206}Pb/^{204}Pb$ ratio in their blood is consistent with that of much of the lead in paint used in the past on Australian houses (*35*). Such changes, especially in isotopic compositions, associated with renovation of leaded paint were documented earlier by Manton (*36*). Despite the changes associated with renovation, the increases in isotopic composition that occur while taking calcium carbonate are consistent with the increases noted above.

One control subject (1302) was a regular firearms shooter, and large changes in PbB and isotopic composition are consistent with lead exposure from this activity. His data were omitted from the discussion and will be documented separately.

A husband and wife control pair (1322, 1323) exhibited small changes in PbB and isotopic composition after an 11-day visit to the United States (Table 2). High $^{206}Pb/^{204}Pb$ ratios are characteristic of U.S. subjects and many environmental and dietary media (*39*).

Statistical analyses. We omitted the data for the anomalous subjects 1302, 1305, and 1306 from the statistical analyses. Significant interactions between group and treatment were found for all blood lead isotopic ratios (*p*-values for *F*-ratios with 2 and 14 degrees of freedom ranged from 0.01 for $^{208}Pb/^{206}Pb$ through 0.023 for $^{207}Pb/^{206}Pb$ to 0.027 for $^{206}Pb/^{204}Pb$). The group-by-treatment interaction was not significant for lead concentrations ($p = 0.402$). We followed up the significant interactions by testing interaction contrasts to answer the question, does the change between baseline and treatment differ for different pairs of groups? Two contrasts compared the calcium carbonate product and the complex calcium citrate product to the controls. The third contrast compared the complex calcium citrate product to the calcium carbonate product. The results are listed in Table 3. Using an adjusted α level of 0.0167 (0.05/3) to allow for the three contrasts, there was a significant difference in the change from baseline and treatment for the calcium carbonate product and the control group for the $^{208}Pb/^{206}Pb$ ratio ($p = 0.008$) and close

Table 2. Changes in lead isotopic composition and blood lead for subjects.

			Pretreatment		Changes during treatment		Posttreatment sample		
Identifier	Age (years)	Sex	PbB (µg/dL)	$^{206}Pb/^{204}Pb$	PbB (µg/dL)	$^{206}Pb/^{204}Pb$	PbB (µg/dL)	$^{206}Pb/^{204}Pb$	Compliance[a] (%)
Calcium citrate/phosphate/chelate									
1303	31	M	3.5	17.19	3.2–2.9	17.21–17.25	—	—	70–100
1304	38	M	2.1	17.22	2.1–1.4	17.22–17.32	—	—	87–94
1309	34	M	1.4	17.21	1.3–1.4	17.19–17.34	—	—	73–78
1311	47	F	5.8	16.66	5.2–5.7	16.68–16.70	—	—	76–90
1313	21	F	2.5	17.32	2.3–2.6	17.24–17.33	—	—	0–50
1314	30	F	3.1	17.49	3.1–3.8	17.42–17.52	—	—	21–59
1315	44	F	2.2	17.22	1.5–1.4	17.24–17.28	—	—	86–92
1321	27	F	1.8	17.45	1.5–1.5	17.36–17.54	—	—	29–40
Calcium carbonate									
1305[b]	39	M	4.5–7.8	16.76–16.39	6.9–4.5	16.48–16.84	4.1	16.88	94–100
1306[b]	36	F	2.2–3.5	17.36–16.93	2.2–1.9	17.25–17.46	1.9	17.53	74–90
1308	32	F	3.5–4.3	16.84	5.1–3.3	17.31–17.42	2.6	17.34	70–89
1312	25	F	2.1	17.39	2.3–2.1	17.49–17.64	1.6	17.51	84–96
1316	27	F	1.5	17.52	1.4–1.5	17.63–17.70	1.3	17.58	70–86
1319	33	F	2.1	17.35	2.0–2.6	17.32–17.47	2.1	17.38	17–62
1320	30	F	1.2	17.28	1.2–1.4	17.36–17.38	1.0	17.33	29–40
Controls									
1302[b]	38	M	3.2–6.7	17.39–17.10	3.8–6.0	17.58–17.21	—	—	—
1307	32	M	2.3	17.26	2.9–2.1	17.23–17.24	—	—	—
1317	23	F	1.8	17.37	1.5	17.37–17.38	—	—	—
1318	39	M	1.7	17.20	1.3–1.5	17.21–17.26	—	—	—
1322[b]	25	F	2.4	17.24	2.3–1.8	17.54–17.35	—	—	—
1323[b]	31	M	2.4	17.36	2.6–2.2	17.43–17.35	—	—	—

[a]Compliance each month over the 3 months of treatment. [b]Possible reasons for the larger variability in isotopic composition and lead concentration are given in the text.

to significant differences for the $^{207}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ ratios ($p = 0.028$ and 0.030, respectively). In contrast, there was no suggestion of significant differences between the control and calcium citrate groups ($p > 0.5$ for each measure). The third contrast showed that the change for calcium carbonate product differed significantly from that for the calcium citrate product for each of the three measures ($p < 0.010$ in each case). The overall result was that the change from baseline to treatment for the calcium carbonate group differed from that for both the control and calcium citrate groups.

Discussion

Contribution to blood of lead from supplements. Over the 6-month dosing period, the potential impact on blood lead from the two calcium supplements tested in this pilot study was not detectable in the case of the complex product and only a small contribution was detected in the case of the calcium carbonate product. For the calcium carbonate product, there was little or no change in blood lead concentration but detectable changes in isotopic composition toward the $^{206}Pb/^{204}Pb$ value of 19.7 in the supplement. Changes in isotopic composition toward the contributing source but not in blood lead concentration were observed in some other studies on newborn infants (*37*) and percutaneous absorption of lead (*38*). This may indicate that the pharmacokinetics of lead in blood need to be reevaluated, as also indicated by the long half-lives of lead in blood of up to 38 months observed in several young infants unnaturally exposed to lead from home renovations (*39*). With the changes in isotopic composition in blood compared with those in the pretreatment phase of calcium carbonate relative to the values in the product, the maximum contribution to blood lead is < 0.5%.

Indirect evidence supporting the protective effect of calcium (calcium carbonate ± phosphorous) comes from our longitudinal investigation on mobilization of lead from the maternal skeleton during pregnancy and lactation. In four pregnant subjects who were given calcium supplements (calcium carbonate or calcium citrate from the present study), minimal changes in blood lead concentration and isotopic composition were observed during pregnancy and lactation (*40*).

Effect of type of supplement. Given the competition of calcium and lead, an additional factor contributing to the uptake of lead from the gastrointestinal tract is the relative absorption of calcium from different compounds. The absorption of calcium from calcium carbonate and calcium citrate alone is approximately 30% (*41*). The absorption may be higher for the complex product of calcium citrate/phosphate/chelate, but to our knowledge this has not been rigorously tested using isotopic tracing methods.

Effect of state of the gut. Another important factor affecting gastrointestinal absorption is the relative condition of the gut—that is, whether it is in a fasted or nonfasted state. Tracer studies have demonstrated that in the absence of additional calcium, gastrointestinal absorption of lead was greater when lead was ingested by fasting subjects given soluble lead compounds in distilled water than when lead was ingested in the presence of food. Lead absorption in nonfasted subjects on either a normal diet or a controlled diet, in which nutritional content was known, ranged from approximately 3% to 10%, whereas lead absorption in fasted subjects who received soluble lead in distilled water (with no additional calcium) ranged from approximately 30% to 76% (*20–24,33,34*). The absence of calcium and other minerals in the gastrointestinal tract at the time of lead ingestion is a major reason for increased lead absorption in fasting subjects compared with nonfasting subjects (*20–24,33,34*), although when calcium and other minerals are present, differences seen between fasting and nonfasting subjects essentially disappear (*22,23,32*).

Effect of minerals on absorption. The presence of other minerals than calcium is an important factor in lead absorption from the gastrointestinal tract. For example, lead absorption decreases as calcium (± phosphorous) concentrations increase (Figure 1) (*20,22*). Lead absorption in fasting subjects given lead chloride in drinking water was reduced by 83% and 97% when the lead was given with calcium and phosphorous at intakes of 200 mg Ca + 140 mg P and 700 mg Ca + 500 mg P, respectively (*22*). Reductions in lead absorption and retention were noted with both calcium alone (as calcium carbonate) and phosphorous alone (as sodium phosphate), but calcium was much more effective than phosphorous (*20,22*). The larger decreases in absorption for calcium phosphate compared with calcium carbonate observed by Blake and Mann (*20*) (Figure 1) may explain the absence of any effect of the complex product used in our study.

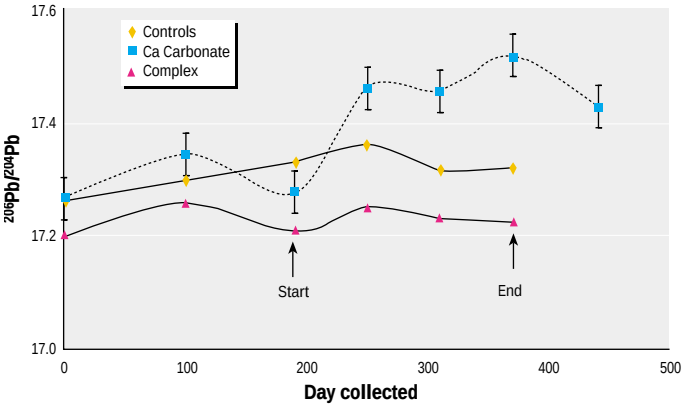

Figure 2. Variation in mean $^{206}Pb/^{204}Pb$ ratio for the different groups over time. The $^{206}Pb/^{204}Pb$ ratio was 19.7 in the calcium carbonate supplement and 20.14 in the complex supplement, so any contribution from these supplements to blood lead should be easily discernible. Beginning of the administration of the supplement is denoted by "Start" and termination by "End." An additional sample posttreatment was taken for the calcium carbonate supplement to verify its potential effect on the blood lead of subjects. The data for the "anomalous" subjects 1302, 1305, and 1306 are not included in the analysis. The error bars indicate the maximum SE measured at each stage.

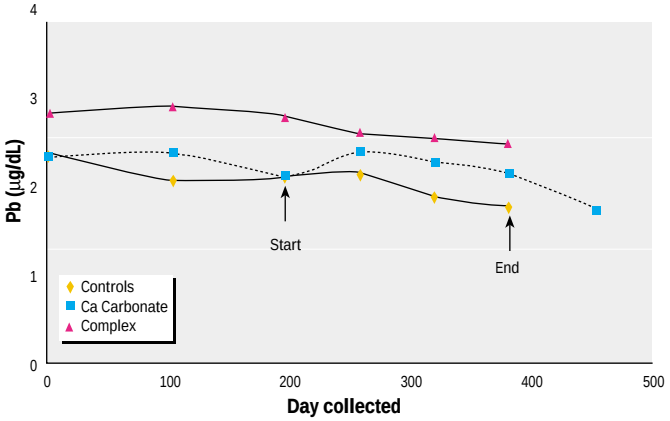

Figure 3. Variation in PbB (µg/dL) for the different groups over time. The daily intake of lead from the supplements is about 3 µg Pb. Beginning of the administration of the supplement is denoted by "Start" and termination by "End."

Lead absorption and retention among infants and young children is also influenced by dietary calcium intake. For example, Ziegler et al. (*42*) observed an inverse relationship between dietary calcium and retention and lead absorption in young infants in metabolic balance studies. Dietary calcium and phosphorus were important predictors of blood lead concentrations in children 12–47 months of age from a low-income population in central Washington, D.C. (*43*). Likewise, Sorrell et al. (*44*) and Johnson and Tenuta (*45*) observed inverse correlations between lead and calcium intake, vitamin D, and milk-based foods. Reductions in lead absorption were also noted in subjects ingesting different foods, depending on the calcium and mineral content of the ingested meal (*24*).

Animal studies. Studies using fasted and nonfasted laboratory animals, including rats, mice, and monkeys, have produced results similar to those in humans (*46–49*). For example, Mahaffey-Six and Goyer (*46*) and Mahaffey et al. (*47*) reported that rats ingesting a low-calcium diet had blood lead concentrations approximately 4 times higher than rats consuming a normal calcium diet. Later studies showed that gastrointestinal absorption of lead decreases in the presence of a number of minerals (*50–52*). Increasing calcium concentrations were found to decrease lead retention exponentially as calcium concentrations increased (*49*). As in the human studies, administration of phosphorous without calcium did not produce reductions in lead retention as great as that for calcium alone or for calcium with phosphorous (*50*).

Effect of dosing regime. An additional protective effect associated with the complex product may come from its dosing regime. This supplement was to be taken 4 times daily including at bedtime because the fasting state is a time when most parathyroid hormone-mediated bone resorption occurs (*26*). The calcium carbonate was taken twice daily after meals. Heaney (*26*) recommended a daily dosing regime of 4 times because, although the amount of a mineral absorbed continues to rise with the intake, the percentage absorbed falls.

Matrix effects. The minimal effects observed in our pilot study may also be caused partly by matrix effects of the products. In studies of fasted human subjects, lead was administered as dissolved salts or suspended in solution. Using absorption values for lead in a solution as representative of lead absorption from a complex matrix may produce an overestimate of lead absorption. Before absorption, lead must dissociate from the compound to which it is bound. This may explain the difference between the complex calcium citrate and the calcium carbonate; the latter was in a chewable form and more easily absorbed.

Effect of duration of investigations. The length of time over which a study is undertaken may also be an important factor in absorption of lead from the gastrointestinal tract. Unlike the investigations by Rabinowitz et al. (*23,33*), the other studies involving radioactive tracers were only of short duration—less than 7 days. Our treatment period was 6 months, but we were able to detect increases in isotopic composition for calcium carbonate supplement. Because calcium carbonate is the most popular supplement (*53*), the potential uptake of lead from this product should be verified in a more comprehensive investigation, especially of young children.

Daily intake of lead from supplements. The amounts ingested from the two supplements of approximately 3 µg Pb/day are more than half the current daily lead intake of less than 5 µg Pb/day for the United States (*19*)—despite the temporal decrease in the amount of lead in supplements illustrated by Scelfo and Flegal (*15*). Lanphear and colleagues recently demonstrated that lead shows detrimental effects in children at blood concentrations as low as 2.5 µg/dL (*54*). Hence, any source contributing 50% or more to lead exposure—especially in young children, with lead absorption greater than that of adults—requires investigation and monitoring.

In conclusion, despite the fact that lead exposure from the calcium supplements tested in this study was approximately half the daily intake of lead, the contribution to blood lead concentration was minimal. However, the same may not apply to calcium-fortified foods. Furthermore, even though there is no discernible increase in blood lead concentration, there are significant changes in the isotopic composition of lead in blood arising from the calcium carbonate supplement, which indicate a limited input of lead from diet into the blood. Because calcium carbonate is the overwhelmingly most popular calcium supplement, the changes we have observed merit further investigation. The small contributions of lead from calcium supplements, however, should not discourage people from their usage given other significant benefits deriving from calcium.

Table 3. Interaction contrasts.

Analysis	Coefficient	SE	*t*-Value	*p*-Value
$^{208}Pb/^{206}Pb$				
Calcium carbonate vs. control	–0.01692	0.00548	–3.0898	0.00799
Calcium citrate vs. control	–0.00011	0.00465	–0.02456	0.98075
Calcium citrate vs. calcium carbonate	–0.01681	0.00500	3.3618	0.00465
$^{207}Pb/^{206}Pb$				
Calcium carbonate vs. control	–0.01015	0.00414	–2.4480	0.02815
Calcium citrate vs. control	–0.00155	0.00352	0.4405	0.66631
Calcium citrate vs. calcium carbonate	0.01169	0.00378	3.0918	0.00796
$^{206}Pb/^{204}Pb$				
Calcium carbonate vs. control	0.22127	0.09147	2.4190	0.02977
Calcium citrate vs. control	–0.02776	0.07774	–0.35711	0.72633
Calcium citrate vs. calcium carbonate	–0.24903	0.08350	–2.9823	0.00989

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