Skeletal Lead Release during Bone Resorption: Effect of Bisphosphonate Treatment in a Pilot Study

Brian Gulson,[1,2] Karen Mizon,[1,2] Howard Smith,[3] John Eisman,[4] Jacqueline Palmer,[2] Michael Korsch,[2] John Donnelly,[5] and Kay Waite[3]

[1]Graduate School of the Environment, Macquarie University, Sydney, New South Wales, Australia; [2]CSIRO/EM, North Ryde, New South Wales, Australia; [3]Department of Endocrinology, Westmead Hospital, Sydney, New South Wales, Australia; [4]Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, New South Wales, Australia; [5]John B Donnelly & Associates Pty Limited, Sydney, New South Wales, Australia

There has been renewed interest in impacts on physiologic systems in the middle and older age groups, especially from fractures and hypertension. Increased blood lead (BPb) levels in postmenopausal females, which are thought to arise from bone demineralization, may also relate to other health effects including hypertension. Taking advantage of natural differences in lead isotope signature between Australian sources of lead and those from other countries, a 2-year pilot study was performed in premenopausal and postmenopausal females and male partners in which the subjects were administered a bisphosphonate, alendronate, for 6 months. The aim of the study was to determine how lead isotopes and lead concentrations changed in relation to bone remodeling processes. Premenopausal subjects were a woman (and male partner) from Bosnia and two women from Colombia. The postmenopausal subject was a woman from Russia. Her male partner and one man from Sri Lanka were included. Multigenerational Australian subjects were 2 perimenopausal women and 1 postmenopausal woman. Each subject had blood and urine samples collected for markers of bone turnover and for lead isotope studies monthly for 7–9 months before, for 3 months during, and for up to 6 months after treatment with alendronate to inhibit bone resorption. Each subject thus acted as his or her own control. As predicted, there were significant decreases in the lead isotope ratio, $^{206}Pb/^{204}Pb$, for the migrant subjects during treatment compared with the pretreatment period ($p < 0.01$). After cessation of treatment, an increasing isotope ratio for the postmenopausal subject (and older male partner) occurred later than for premenopausal subjects, indicative of prolonged efficacy of the alendronate for the older subjects. The average BPb concentrations in migrant subjects decreased by about 20% during the treatment compared with the pretreatment period ($p < 0.01$). To our knowledge, these are the first BPb concentrations reported over monthly to quarterly intervals for environmentally exposed adults over an extended period. The changes in lead isotopic composition and lead concentration are consistent with a decrease in bone resorption and associated mobilization of lead during alendronate therapy. Older subjects at risk of fractures may benefit from treatment with antiresorptive therapy, such as the potent bisphosphonates, with the added bonus of lower release of lead from bones and thus less risk of the potential adverse health effects of increased BPb levels. *Key words:* **bisphosphonate, blood, bone mineral density, bone turnover, isotopes, lead, NTx test.** *Environ Health Perspect* **110:1017–1023 (2002). [Online 27 August 2002]**
http://ehpnet1.niehs.nih.gov/docs/2002/110p1017-1023gulson/abstract.html

Exposure to lead is still an international public health problem, despite major reductions in its use in industrial processes in developed countries (*1*). The neurotoxic effects of lead in the fetus, neonate, and infant are well recognized (*2*). The main reservoir of lead within the body is the skeleton and, until recently, lead was considered to be relatively immobile in this compartment. Recent studies using the stable lead isotope fingerprinting method in nonhuman primates (*3,4*) as well as in humans (*5,6*) indicate that lead, like calcium, is mobilized from the maternal skeleton and transferred to the fetus and neonate during pregnancy and lactation. Other times of physiologic stress that could result in additional release of lead from the skeleton include menopause (*7*). In a preliminary assessment of data from the Third National Health and Nutritional Examination Survey (NHANES III), higher blood lead (BPb) levels were observed in postmenopausal compared with premenopausal women (3.9 vs. 2.6 μg/dL), consistent with the increased bone turnover that occurs during the hormonal changes of menopause. Moreover, higher BPb levels were associated with lower bone density in perimenopausal women (*8*). Similar relationships in BPb and menopause were noted earlier (*9*). In a study of 903 women 35–64 years of age from Mexico City, the highest BPb levels were observed in women 47–50 years of age, with a mean difference between pre- and postmenopausal women of 0.76 μg/dL (*10*). These higher blood levels could have significant health implications because increased BPb levels in adults have been correlated with hypertension (*11–16*), decreased renal function (*17*), impaired neurocognitive function (*18*), and Alzheimer disease (*19*).

Antiresorptive agents that inhibit resorption in the bone remodeling process may reduce or even reverse the demineralization process documented during pregnancy and lactation, observed in perimenopausal women (*20*), and seen in men and women with corticosteroid-induced osteoporosis (*21*). Thus, these agents may have the additional benefit of preventing increases in BPb levels commonly seen in these life stages. During pregnancy, calcium supplementation is associated with lower BPb levels (*22–24*), although calcium given alone has not been proven to reverse the loss of bone mineral density during pregnancy (*24*) or in postmenopausal subjects (*25*). In menopausal women, antiresorptive agents such as hormone replacement therapy (HRT) and bisphosphonates are capable of preventing loss of bone density (*20,25,26*). Postmenopausal women taking HRT have been observed to have significantly higher cortical bone lead concentrations than those not taking HRT (*27*). To our knowledge there have been no prospective studies to document the effect of antiresorptive agents on BPb levels in healthy adults.

We performed a pilot study of the effect of a potent bisphosphonate (alendronate) administered over a 6-month period on BPb levels and other markers of bone turnover in healthy pre- and postmenopausal women and men. The aim of this study was to determine how BPb isotopes and BPb concentrations changed in relation to bone remodeling processes and to changes in bone resorption. We included males in this study because of the concerns raised earlier by the U.S. National Institutes of Health about increased bone fractures in males (*28*).

Materials and Methods

Subjects. Subjects in our main group were immigrants to Australia whose skeletal lead isotopic composition was different from that

Address correspondence to B.L. Gulson, Graduate School of the Environment, Macquarie University, Sydney, NSW 2109, Australia. Telephone: 61 2 9850 7983. Fax: 61 2 9850 7972. E-mail: bgulson@gse.mq.edu.au

We thank M. Salter for phlebotomy, and we thank the participants in this study.

Received 24 January 2002; accepted 14 March 2002.

in their current environment. In essence, the lead isotopic composition or "signature" in multigenerational Australian residents is different from that in subjects from most other countries because the historical sources of lead in Australia are dominated by geologically old lead (*5,6,29*). Hence, by monitoring the BPb isotopes of migrant subjects after arrival in Australia, it was possible to detect changes in isotopic composition and BPb concentration related to mobilization of skeletal lead as was first explored by Manton (*30,31*).

Premenopausal subjects included two women from Colombia and a woman and her male partner from Bosnia. The older group included a postmenopausal Russian woman and her male partner and a male from Sri Lanka. The multigenerational Australian group included three women—two perimenopausal and one postmenopausal—and one male partner, all who were long-term Australian residents. The Australian subjects were included as a comparison with the migrant subjects but have a more complex history of HRT.

Protocols. Before commencing any treatment, we collected blood and urine samples from each subject for measurements of markers of bone turnover. For the lead isotope measurements, seven to nine blood and urine samples were collected monthly before alendronate therapy, monthly during therapy, and on two occasions 3 and 6 months after discontinuing the drug therapy. The bone turnover markers were repeated after 3 months of alendronate therapy (10 mg/day) and again 3 months after discontinuing the drug therapy. Each subject thus acted as his or her own control.

None of the migrant subjects had previously taken HRT. One perimenopausal Australian subject (subject 1112) replaced HRT with alendronate for 6 months during the treatment period. The postmenopausal Australian subject (subject 800) took HRT in addition to alendronate throughout the study.

A questionnaire based on that developed by the Bone and Mineral Research Program at the Garvan Institute, and especially focused on calcium intake, was administered on one occasion by the cohort coordinator (K.M.) at the beginning of the study. Any major changes in dietary intake were examined at each sampling time point.

Ethics approval was obtained from the Western Sydney Area Health Service, Human Research Ethics Committee.

Analytical methods. All sample preparation was performed in purpose-built low-contamination laboratories ("clean rooms") incorporating 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 < 1 g removed to a clean Teflon vessel. We added a ^{202}Pb spike solution of known isotopic composition and lead concentration (~10 ng/g) to the aliquot to obtain the concentration of lead and isotopic composition of the unknown sample in the one analysis, the isotope dilution method. ^{202}Pb, with a half-life of about 3×10^5 years, is not naturally occurring but is a cyclotron by-product of preparation of thallium. We further separated lead from interfering ions such as iron and zinc using anion exchange chromatography in a bromide medium.

For isotope ratio measurement, we loaded fractions of the purified lead samples onto a zone-refined 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 Isotopes, Winsford, 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 estimated precision for the isotopic ratios is ± 0.2% (2 σ) on the ^{206}Pb/^{204}Pb ratio, ± 0.1% on the ^{208}Pb/^{206}Pb and ^{207}Pb/^{206}Pb ratios, and ± 3% for the lead concentration. Data were normalized to the accepted values of the international standard NIST SRM 981 (National Institute of Standards and Technology, Gaithersburg, MD, USA) by applying a correction factor of + 0.08% per atomic mass unit to allow comparisons between laboratories. A measurement of the environmental lead acquired by the sample throughout the entire preparation analysis procedure was obtained in the form of a lead blank measurement. The amount of contamination detected in blanks was generally around 200 pg for blood. As the blanks contributed negligibly to the lead in the sample, no blank corrections were performed.

Biochemical markers of bone turnover. The NTx test is an enzyme-linked immunosorbent assay of urinary cross-linked N-telopeptides of type 1 collagen, which are specific to bone and are stable in urine (OSTEOMARK, Ostex International, Seattle, WA, USA). Clinical studies have demonstrated reductions in the NTx value during HRT in postmenopausal women (*25*) and after treatment with alendronate (*26*). Serum measurements included calcium, phosphate, total alkaline phosphatase, and osteocalcin. We measured bone mineral density by dual-energy X-ray absorptiometry (Norland XR 36 Densitometer) (Xtron Imaging Inc., Mississauga, Ontario, Canada) at the lumbar spine, femoral neck, and wrist on two occasions 12 months apart, one before alendronate treatment and the other after treatment. To assess bone density, we used the T-score, which compares the measured bone density with that of healthy young adults, and the Z-score, which compares the measured bone density of the subject with the average of persons of the same age.

Statistical analysis. Using the same statistical methods as described by Gulson et al. (*6*), we fitted separate regression lines by least squares for each subject in each of the three periods before, during, and after alendronate therapy. This enabled the comparison of the average rate of change of blood measurements

Table 1. Information on subjects.

Subject	Country of origin	Sex/age	Menopausal status	Time in Australia (days)	First BPb concentration (μg/dL)	Bone mineral density					
						Lumbar spine		Femoral neck		Wrist	
						Z	T	Z	T	Z	T
1101	Bosnia	M/54	—	678	3.6	0.9	0.3	0.8	–1.2	0.5	0.3
1102	Bosnia	F/44	Pre	678	1.4	2.26	1.51	0.06	–0.72	1.48	1.52
1103	Colombia	F/45	Pre	558	3.9	0.1	–0.7	0.8	–0.1	0.1	0.1
1104	Colombia	F/46	Pre	396	1.4	0.02	–0.79	0.18	–0.67	–0.72	–0.68
1105	Bulgaria	M	—	27	10.6	NM	NM	NM	NM	NM	NM
1106	Bulgaria	F	Post	27	7.9	NM	NM	NM	NM	NM	NM
1107	Russia	M/67	—	269	3.9	1.7	0.9	0.9	–1.7	2.5	2.0
1108	Russia	F/70	Post	269	1.5	0.79	–0.9	–0.7	–2.9	–1.7	–2.1
1110	Australia	F/57	Peri	—	6.0	1.1	–0.1	3.3	1.9	0.6	0.4
1112	Australia	F/53	Peri	—	8.9	2.5	1.4	0.1	–1.0	1.7	1.7
1113	Sri Lanka	M/57	—	220	3.2	–0.2	–0.8	1.5	–0.5	–0.2	–0.5
800	Australia	F	Post	—	3.3	–0.01	–0.97	–2.3	–3.35	–0.98	–0.98

Abbreviations: F, female; M, male; NM, not measured (discontinued trial).

for the three periods. Where there was no significant increase or decrease ($p > 0.05$) within a period, a horizontal line was plotted through the mean for each period for each subject. We used analysis of variance (ANOVA) to compare differences in average response between periods for the perimenopausal group. For comparisons within the perimenopausal group, the analysis was repeated with male subject 1101 omitted. Similar analysis could not be performed on the postmenopausal group because of small numbers of subjects in different categories.

Results

Information about the subjects is provided in Table 1 along with the subjects' BPb concentrations at the first sampling and their bone mineral densities. The statistical results from the regression analyses of BPb concentration and $^{206}Pb/^{204}Pb$ ratio for the three monitoring periods are shown in Table 2, and the ANOVA results are shown in Table 3.

To our knowledge, these are the first longitudinal BPb measurements undertaken on elderly subjects over an extended length of time. For example, only two BPb measurements 3 years apart were measured in the Normative Aging Study (*32*), and only one measurement was taken in the Swedish Twin Registry (*33*).

Hypothetical response for lead. Before undertaking this study and using information from earlier and ongoing studies, we predicted the changes that could have been observed in BPb isotopic composition and concentration during alendronate therapy. The hypothetical responses for menopausal migrant and multigenerational Australian subjects are shown in Figure 1. As six of the eight migrant subjects had resided in Australia for more than 6 months, lead in the blood should have reached a steady state between skeletal lead and environmental (Australian) lead, similar to that demonstrated earlier (*5,6,28*). During administration of alendronate there should be a decrease in the $^{206}Pb/^{204}Pb$ ratio (Figure 1; or an increase in $^{207}Pb/^{206}Pb$ ratio) and possibly a decrease in BPb concentration, assuming this drug inhibits bone resorption. Such changes would reflect a greater input to the isotopic composition of exogenous Australian lead such as diet, air, water, and house dust lead compared with that from skeletal tissues. A decrease in BPb concentration would reflect a decrease in the amount of lead being released from skeletal tissues to blood, rather than any changes in excretion or clearance of the lead from the blood (*34*). After cessation of the treatment and a resumption of bone resorption, the $^{206}Pb/^{204}Pb$ ratio and possibly the BPb concentration should rise, reflecting increased mobilization of lead from the skeleton (Figure 1). As the $^{206}Pb/^{204}Pb$ ratio in multigenerational Australian subjects is less than 17.1 and closer to 16.5 in older Australians (*35*), there should be limited changes in lead isotopic composition and BPb concentration for the Australian subjects, with a possible increase in $^{206}Pb/^{204}Pb$ ratio during treatment and a decrease in $^{206}Pb/^{204}Pb$ ratio after treatment. The BPb concentration could be expected to decrease during treatment to an extent related to the total skeletal lead burden and the underlying rate of bone turnover.

Baseline monitoring for lead. The BPb concentrations in the first sample obtained from the migrant subjects ranged from 1.4 to 10.6 µg/dL (Table 1), surprisingly low given the purported environmental contamination in their country of origin and their relatively long exposure to lead compared with current levels. The highest values were for the Bulgarian couple, who returned to Bulgaria during the trial. The BPb concentrations at the time of initial sampling for the three Australian subjects ranged from 3.3 to 8.9 µg/dL (Table 1). These BPb concentrations are generally below the level of concern of 10 µg/dL promulgated by the U.S. Centers for Disease Control and Prevention (*36*). The data for the baseline monthly measurements (Figures 2 and 3) of BPb isotopic composition and lead concentration exhibited a larger variation than we expected based on our previous work. For example, there are only small variations for premenopausal migrant adult females of child-bearing age who did not conceive in the pregnancy study (Figures 4 and 5) (*37*) or even pregnant subjects in our current pregnancy cohort who took calcium supplements (*38*). The variations were largest in BPb concentration for subjects 1101, 1104, and 1110 and in isotopic composition for most subjects except 1108 (Table 2; Figures 2 and 3).

Treatment period compared with pretreatment period. Compared with the pretreatment measurements, the data for the treatment period showed consistent decreases in mean levels for BPb isotopic composition and lead concentration for most subjects (Table 2; Figures 2 and 3). A significant decrease of about 20% in average BPb concentration ($p < 0.01$) was observed for all migrant subjects

Table 2. Statistical results for BPb concentration and $^{206}Pb/^{204}Pb$ from regression analyses.

	Before treatment		During treatment		After treatment[a]	
Subject	R^2	*p*-Value	R^2	*p*-Value	R^2	*p*-Value
BPb concentration						
1101	0.089	0.40	0.57	0.08	0.20	0.56
1102	0.38	0.10	0.58	0.08	0.08	0.71
1103	0.44	0.05	0.68	0.09	0.83	0.09
1104	0.10	0.34	0.35	0.29		
1107	0.18	0.26	0.94	0.01	0.64	0.20
1108	0.60	0.04	0.56	0.05	0.25	0.50
1110	0.07	0.54	0.09	0.62	0.82	0.10
$^{206}Pb/^{204}Pb$						
1101	0.11	0.34	0.30	0.26	0.59	0.23
1102	0.05	0.58	0.17	0.41	0.36	0.40
1103	0.06	0.52	0.92	0.01	0.28	0.47
1104	0.03	0.59	0.58	0.13		
1107	0.14	0.32	0.88	0.02	0.73	0.15
1108	0.59	0.04	0.36	0.15	0.61	0.22
1110	0.00	0.99	0.58	0.13	0.96	0.02

[a]Because sample size is based on 1 and 2 degrees of freedom, it is inadequate for significance testing.

Table 3. Average response for the premenopausal group.

Response	Before treatment	During treatment	After treatment
Blood Pb, all subjects (µg/dL)	2.14	1.74*	1.71 (NS)
Blood Pb, omitting subject 1101	1.83	1.46*	1.45 (NS)
$^{206}Pb/^{204}Pb$ ratio, all subjects	17.67	17.57*	17.55 (NS)
$^{206}Pb/^{204}Pb$ ratio, omitting subject 1101	17.73	17.62*	17.61 (NS)
$^{207}Pb/^{206}Pb$ ratio, all subjects	0.8807	0.8850*	0.8860 (NS)
$^{207}Pb/^{206}Pb$ ratio, omitting subject 1101	0.8777	0.8827*	0.8830 (NS)

NS, no significant difference between during and after treatment.
*Significant difference between during and before treatment ($p < 0.01$).

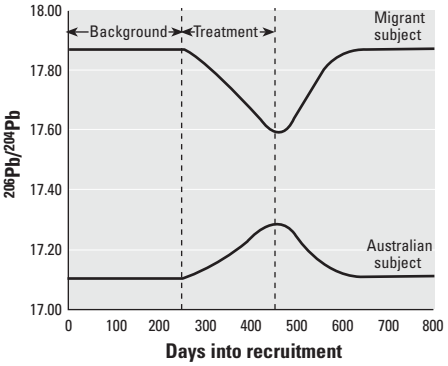

Figure 1. Predicted trends for changes in the $^{206}Pb/^{204}Pb$ ratio for a migrant subject and a long-term Australian subject before alendronate treatment, during treatment, and after treatment.

(Tables 2 and 3). The $^{206}Pb/^{204}Pb$ ratio was also significantly reduced ($p < 0.01$). The same results were obtained after omitting the male subject 1101 from the analyses (Table 3). For the three premenopausal female subjects (1102, 1103, 1104), there was a decrease in average $^{206}Pb/^{204}Pb$ ratio of about 0.6%, compared with our measurement error of 0.2% (e.g., Figure 4). Compared with the premenopausal subjects, the female postmenopausal migrant, subject 1108, exhibited a slightly larger decrease in $^{206}Pb/^{204}Pb$ ratio of up to 1.6% and a decrease of approximately 17% in BPb concentration during the treatment phases relative to the pretreatment phase.

The male subjects 1101 and 1107 exhibited changes in $^{206}Pb/^{204}Pb$ ratio and BPb concentration similar to their partners, 1102 and 1108, respectively (Table 2; Figures 2 and 3).

Post-treatment. One of the premenopausal subjects (1104) could not be followed post-treatment because only one sample was available. As mentioned above, the hypothetical response post-treatment should be an increase in the $^{206}Pb/^{204}Pb$ ratio. In fact, an increase in $^{206}Pb/^{204}Pb$ ratio occurred at different times depending on menopausal status. For the premenopausal subjects, the change in slope for increasing $^{206}Pb/^{204}Pb$ ratio occurred within approximately 60 days from cessation of treatment. In contrast, the blood of the female postmenopausal subject and her partner (1108, 1107) continued to exhibit a decrease in $^{206}Pb/^{204}Pb$ ratio post-treatment, and the change in slope did not occur for more than 150 days of cessation of treatment. The change in slope for the $^{206}Pb/^{204}Pb$ ratio was apparent within 60 days of cessation of treatment in the male subject (1113) who ceased alendronate treatment after only 3 months because of digestive complaints.

Australian subjects. Of the Australian subjects, two were perimenopausal (subjects 1110 and 1112) and one (subject 800) was postmenopausal (Table 1). The statistical results for the Australian subjects 1112 and 800 have not been listed because they showed exceedingly large variations in lead isotopic composition and lead concentration (Figures 2 and 3). For subject 1110, there was a small increase in the $^{206}Pb/^{204}Pb$ ratio but large fluctuations in the BPb concentration during the whole trial (Table 2). The small decrease in the $^{206}Pb/^{204}Pb$ ratio post-treatment was expected because this subject had relatively high BPb concentrations, and analyses of her teeth gave a low $^{206}Pb/^{204}Pb$ ratio of 16.5; teeth provide evidence for the isotopic composition of the skeleton (*35*). There were large fluctuations in isotopic composition for the postmenopausal subject 800, who had been on HRT and alendronate throughout the trial. Subject 1112 was undergoing HRT at the beginning of the trial. At 184 days she discontinued the therapy for 6 months. After she discontinued the therapy, there was an increase in the $^{206}Pb/^{204}Pb$ ratio in her blood and an increase of 25% in BPb concentration, after which it returned to a baseline level.

Bone density. We used the T-score and Z-score to assess bone density. The T-score

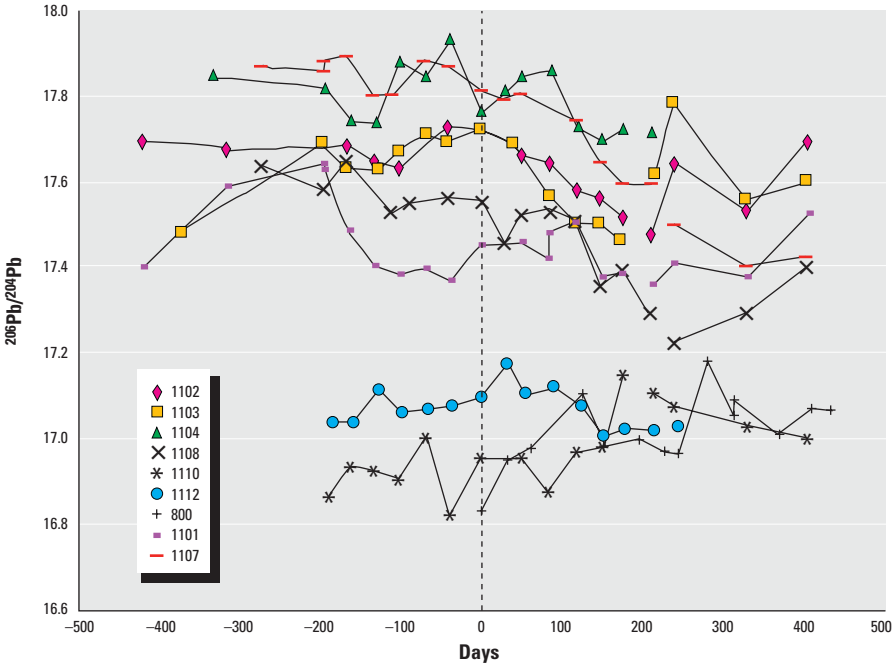

Figure 2. Time-series plots of $^{206}Pb/^{204}Pb$ ratios for migrant subjects (1102, 1103, 1104, 1108, 1101, and 1107) and Australian subjects (800, 1110, and 1112) who underwent alendronate therapy. The therapy began at day 0, and cessation of therapy is represented by a break in the individual time lines. Premenopausal subjects were 1102, 1103, 1104, and 1110; postmenopausal subjects were 1108, 1112, and 800; male subjects were 1101 and 1107. Australian subject 800 was on HRT for the whole study.

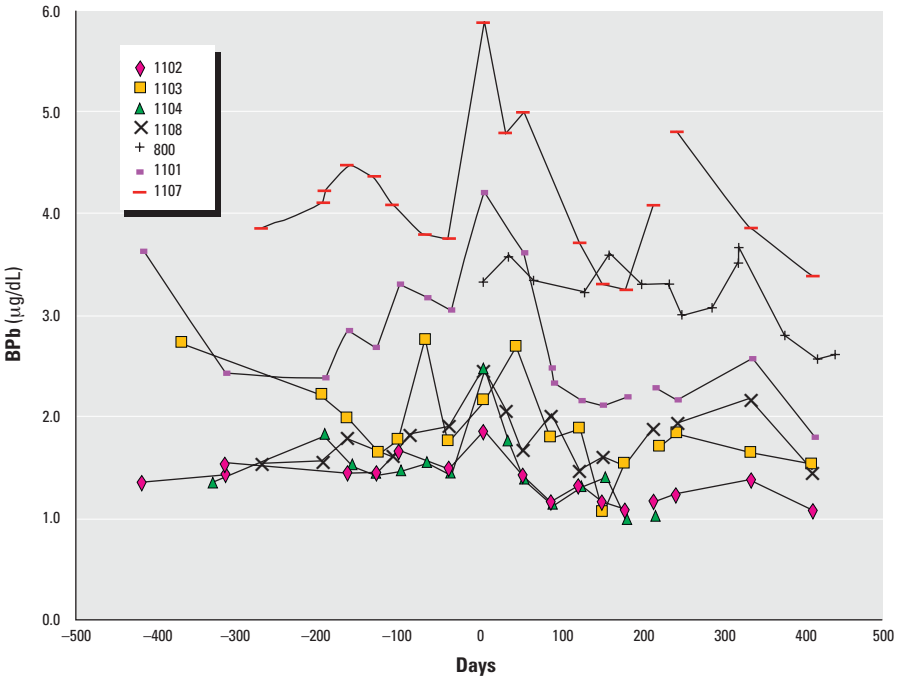

Figure 3. Time-series plots of BPb concentration for migrant subjects (1102, 1103, 1104, 1108, 1101, and 1107) and Australian subjects (800, 1110, and 1112) who underwent alendronate therapy. Therapy began on day 0, and cessation of therapy is represented by a break in the individual time lines. Premenopausal subjects were 1102, 1103, 1104, and 1110; postmenopausal subjects were 1108, 1112, and 800; male subjects were 1101 and 1107. Australian subject 800 was on HRT for the whole study. The BPb concentrations were 5.1–11.1 µg/dL for subject 1110 and 7.1–13.4 µg/dL for subject 1112 and thus are not shown.

describes how the measured bone density compares with that of healthy young adults, and the Z-score compares how the measured bone density compares with the average of persons of the same age as the subject being tested. For a person with a low bone mass (osteopenia) at the spine or hip, the T-score is between –1 and –2.5; for normal bone mass, the T-score is > –1; and for a subject with osteoporosis, the T-score is ≤ –2.5. Overall, the bone density results showed no significant differences for the 12-month period between measurements, given that the precision of the measurement of bone mineral density is 2–5%, although the changes for lumbar spine are probably larger than for the wrist and femoral neck. Subjects 1101, 1102, 1110, and 1112 had normal bone densities. Subjects 1103 and 1104 showed osteopenia in the lumbar spine, with normal wrist and femoral neck; subject 1107 showed osteopenia in the femoral neck; subject 1108 showed osteoporosis in the wrist and femoral neck; and subject 800 showed osteoporosis in the femoral neck and osteopenia in the wrist.

Bone turnover indices. The most sensitive biochemical tests we have that reflect changes in bone resorption are measures of collagen fragments as measured by the NTx assay. The results are reported as a ratio with creatinine to correct for variations in body mass between individuals. In the female migrant subjects, percentage decreases in the NTx/creatinine ratio during antiresorptive treatment relative to pretreatment varied from –16 to –60%, with largest decrease for the postmenopausal subject 1108 (Figure 6). Decreases in the NTx/creatinine ratio were observed for the younger migrant male (subject 1101), but there was an unusual positive trend for the 67-year-old male (subject 1107). Two Australian subjects, who were on HRT before or throughout the study (subjects 800, 1112), exhibited minimal changes in the NTx/creatinine ratio. If only the data for four migrant women are considered, there is a strong correlation (Figure 7; R^2 = 0.94, p = 0.03) between the percentage change from pretreatment to treatment in NTx/creatinine ratio and the percentage change in the lead isotopic ratios.

Lead in blood and initial BPb concentration. Because there is a varying relationship between lead in blood and lead in bone (*10,11,15,17*), the cumulative total of lead in blood over the pretreatment and treatment phases was plotted against the BPb concentration at the time of first sampling. There was a significant correlation in these parameters (Figure 8; $p < 0.001$), with the treatment phase showing lower cumulative amounts of lead in blood compared with the pretreatment phase. This strong correlation contrasts markedly with the scatter of data for the total amount of lead in blood during pregnancy and postpartum versus initial BPb concentration for women of child-bearing age (*5,6*). The difference in the plots may be partly due to the smaller range in BPb concentrations of the pregnant subjects.

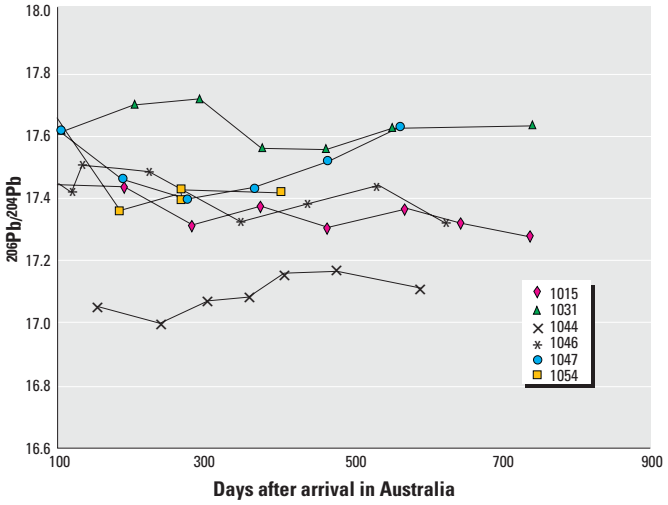

Figure 4. Time-series plots of the $^{206}Pb/^{204}Pb$ ratio for nonpregnant female adults who migrated to Australia. Values were relatively stable after equilibrium was reached between skeletal lead and environmental lead.

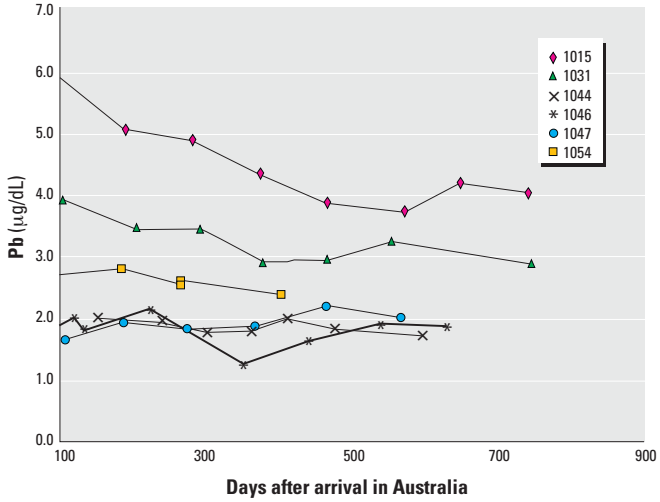

Figure 5. Time-series plots of BPb concentration for nonpregnant female adults who migrated to Australia. Values were relatively stable after equilibrium was reached between skeletal lead and environmental lead.

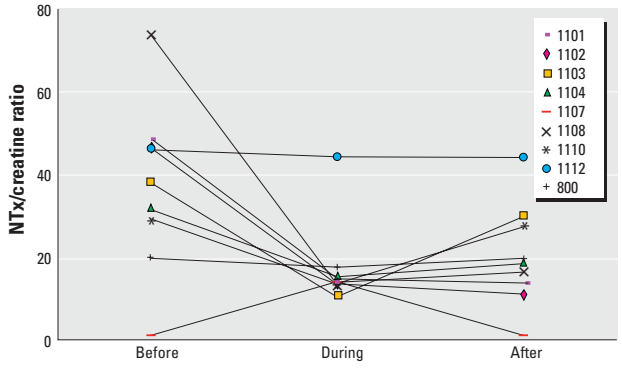

Figure 6. NTx/creatinine ratio in subjects before, during, and after antiresorptive therapy (alendronate).

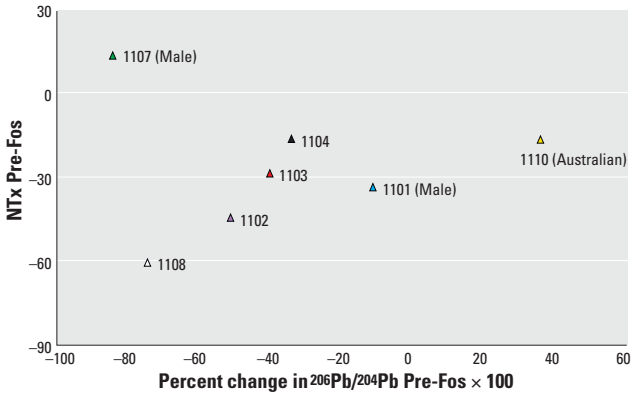

Figure 7. Percentage change from pretreatment to treatment (Pre-Fos) in NTx/creatinine ratio and the percentage change in lead isotopic ratios. This figure suggests that the more bone response in terms of resorption, the more the change in blood from the flux of lead from the bone.

Discussion

In spite of the variability in the data for each subject, the decreases in the BPb concentration and the $^{206}Pb/^{204}Pb$ ratio during antiresorptive treatment followed the hypothetical response. We observed these trends in the premenopausal females (subjects 1102, 1103, 1104) as well as in the postmenopausal female and her older male partner (subjects 1108, 1107). Even though the treatment period of 6 months was relatively short in this study, the effect of the alendronate on bone resorption, as shown by the increasing $^{206}Pb/^{204}Pb$ ratios after treatment, appears to extend for several months after cessation of treatment. The change of increasing $^{206}Pb/^{204}Pb$ ratios for the postmenopausal subject and her male partner occurred later than for the premenopausal subjects and may reflect prolonged efficacy of the alendronate therapy in the older subjects. There was a good correlation for the changes in NTx/creatinine ratio and changes in the lead isotopic ratios for the four female migrant subjects from the pretreatment to treatment periods. The changes in BPb in both premenopausal subjects and the postmenopausal subject were consistent with decreased bone resorption and associated mobilization of lead. However, the expected decrease in BPb levels during alendronate treatment should be more pronounced in early postmenopausal women with increased bone resorption relative to premenopausal women.

The decrease in average BPb concentration of the migrants of about 20% over the relatively short period of treatment of 6 months is considerably greater than changes observed in other investigations of menopausal or elderly subjects at the menopausal transition. For example, Webber et al. (*27*) found that women taking HRT over a 4-year period did not show a significant difference in BPb concentrations. The authors suggested that HRT prevents the menopause-associated increase in bone turnover, such that lead would be expected to remain in the skeleton. In contrast, in the Mexico City study, Hernandez-Avila et al. (*10*) found no difference in BPb concentrations between postmenopausal women who used estrogens and those who did not use them. In spite of the changes in BPb concentration observed in earlier studies (*8–10*), the relatively large fluctuations from serial BPb concentration in our subjects indicate that single or cross-sectional BPb measurements could be misleading in terms of evaluating changes in bone turnover.

The variations observed in both isotopic composition and BPb concentration for premenopausal and postmenopausal subjects in the study compared with the smooth trends found in nonpregnant adult females 18–35 years of age (*37*) (Figures 4 and 5) are considered to reflect more extensive bone remodeling in the older subjects and larger fluctuations in BPb concentration in particular. The significant relations between total lead in blood and initial BPb concentration observed for the elderly subjects (Figure 8) compared with the scatter for pregnant subjects was, however, unexpected and at this stage cannot be easily explained.

It has been argued that the majority of lead in blood of adults in steady state is skeletally derived, ranging from 42% to 75% (*5,29–31,39*), with most evidence favoring the upper end of this range. That is, exogenous lead absorbed directly into the blood in real time can be less than circulating lead from bone release. However, both the data for the migrant subjects and for Australian subjects indicate that with suppression of bone resorption during alendronate treatment, diet and other environmental exposures may play an increased role in BPb, perhaps complemented by decreased calcium absorption associated with aging. It is well established that calcium inhibits uptake of lead from the gastrointestinal tract (*40,41*). Heaney et al. (*42*) have shown there is a gradual decrease in calcium absorption from age 35 onward, accompanied by an additional one-time decrease across menopause. Decreased calcium absorption may explain an increased lead absorption in the elderly. Instead of increased lead absorption from diet during the treatment period, an alternative explanation for the changes in lead isotopic composition and BPb concentrations may reflect contributions from the different bone compartments with their different rates of turnover of lead and exchange of lead and calcium between bone compartments and the blood/serum compartment (*43,44*). Lead and calcium on bone surfaces are thought to be exchanged rapidly with those metals circulating in serum and blood, whereas the exchange between the bone interior and bone surface is considerably slower (*43,44*). As the most recently deposited (periosteal) lead should be that with a dominant Australian isotopic composition, there should be a decrease in the $^{206}Pb/^{204}Pb$ ratio.

The low BPb concentrations in the migrant subjects, especially from Eastern Europe, were surprising given the purported environmental contamination in many of these countries and hence potential long-term lead exposure for the subjects resulting in high bone-lead stores. One explanation for the low BPb concentrations is that any earlier high lead concentrations have been flushed from the blood compartment, as the subjects had been in Australia for more than 9 months. Gulson et al. (*29*) showed that higher BPb concentrations in migrant subjects decrease exponentially after arrival in Australia, and an equilibrium or steady state in BPb concentration (and isotopic composition) in the migrants is reached about 4–6 months after arrival in Australia. For example, the BPb concentration in one subject decreased from 20 µg/dL on arrival to about 6 µg/dL after 6 months, although the lead isotopic composition indicated that approximately 70% of the lead in blood was skeletally derived. In spite of current relatively low BPb concentrations, our subjects could still have high bone-lead stores, available for extra release during periods of physiologic stress such as menopause. If the subjects had high bone-lead stores, one might expect a rebound to higher BPb concentrations once treatment with alendronate had ceased, similar to the increases in skeletally derived lead observed in the pregnancy study (*5,6*). In fact, we did not observe this, as the BPb concentrations were lower after treatment than before treatment (Table 3); because the after-treatment monitoring period was relatively short, it is possible that the lower BPb concentrations may reflect ongoing efficacy of the alendronate.

There appears to be a difference in the variability in BPb isotopic composition and concentration in the period before treatment and during treatment, as shown in Figures 2 and 3 and in the results of the regression analyses. This difference is superimposed on the decreasing lead isotopic ratios and lead concentrations during the treatment period for most of the subjects. For example, there is significant variability in the before-treatment phase where R^2 values in both BPb concentration and isotopic composition are low and only reach 0.6 for subject 1108 (Table 2). In contrast, apart from the Australian perimenopausal subject 1110, the R^2 values during the treatment phase are commonly above 0.5. Is this apparent stability in BPb during the treatment phase, although decreasing, related to the alendronate therapy? Such a hypothesis requires follow-up in a larger and longer term study.

There are a number of potential limitations to this study. The small number of

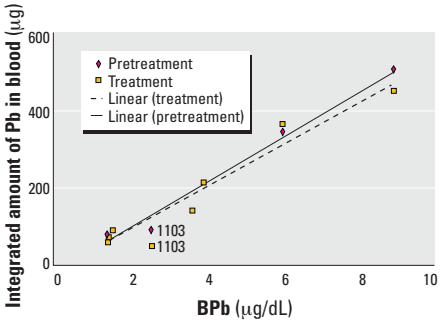

Figure 8. Total amount of lead in blood in the pretreatment and treatment phases compared with the BPb concentration at the time of first sampling. The significant correlation ($p < 0.001$) compared with the scatter observed for pregnant migrant subjects may reflect a higher bone store in the elderly. For linear (treatment), $R^2 = 0.9554$, $p < 0.001$. For linear (pretreatment), $R^2 = 0.9743$, $p < 0.001$.

subjects was exacerbated by withdrawal of several participants for reasons unrelated to the study protocols. Also, the treatment period of 6 months is only about 70% of the full mineralization period of about 40 weeks (*20,45*). However, this study remains the most extensive to date of the effect of antiresorptive agents on BPb concentrations that apparently arise from bone sources. Given the association of BPb levels with potential adverse health outcomes, such as hypertension and dementia, administration of potent antiresorptive drugs such as alendronate to postmenopausal women and older men and women could have additional public health benefits, besides lowering the incidence of fractures in both females and males. The potential effect on other health parameters of antiresorptive agents requires further evaluation. A larger study would allow a quantitative estimate of how much bone remodeling is suppressed by agents such as the bisphosphonates.

References and Notes

1. Thomas VM, Socolow RH, Fanelli JJ, Spiro TG. Effects of reducing lead in gasoline: an analysis of the international experience. Environ Sci Technol 33:3942–3948 (1999).
2. National Academy of Sciences, National Research Council. Measuring Lead Exposure in Infants, Children, Oother Sensitive Populations. Washington, DC:National Academy Press, 1993.
3. Franklin CA, Inskip MJ, Baccanale CL, Edwards CMH, Manton WI, Edwards E, O'Flaherty EJ. Use of sequentially administered stable lead isotopes to investigate changes in blood lead during pregnancy in a nonhuman primate (*Macaca fascicularis*). Fundam Appl Toxicol 39:109–119 (1997).
4. Inskip MJ, Franklin CA, Baccanale CL, Manton WI, O'Flaherty EJ, Edwards CMH, Blenkinsop JB, Edwards EB. Measurement of the flux of lead from bone to blood in a nonhuman primate (*Macaca fascicularis*) by sequential administration of stable lead isotopes. Fundam Applied Toxicol 33:235–245 (1996).
5. Gulson BL, Jameson CW, Mahaffey KR, Mizon KJ, Korsch MJ, Vimpani G. Pregnancy increases mobilization of lead from maternal skeleton. J Lab Clin Med 130:51–62 (1997).
6. Gulson BL, Mahaffey KR, Jameson CW, Mizon KJ, Korsch MJ, Cameron MA, Eisman JA. Mobilization of lead from the skeleton during the post-natal period is larger than during pregnancy. J Lab Clin Med 131:324–329 (1998).
7. Silbergeld EK, Schwartz J, Mahaffey K. Lead and osteoporosis: mobilization of lead from bone in post-menopasual women. Environ Res 47:79–94 (1988).
8. Silbergeld EK. Personal communication.
9. Symanski E, Hertz-Picciotto I. Blood lead levels in relation to menopause, smoking, and pregnancy history. Am J Epidemiol 141:1047–1058 (1995).
10. Hernandez-Avila M, Villalpando CG, Palazuelos E, Hu H, Villalpando MEG, Martinez DR. Determinants of blood lead levels across the menopausal transition. Arch Environ Health 55:355–360 (2000).
11. Hu H, Aro A, Payton M, Korrick S, Sparrow D, Weiss ST, Rotnitsky A. The relationship of bone and blood lead to hypertension. JAMA 275:1171–1176 (1996).
12. Staessen JA, Bulpitt CJ, Fagard R, Lauwerys RR, Roels H, Thijs L, Amery A. Hypertension caused by low-level lead exposure; myth or fact. J Cardiovasc Risk 1:87–97 (1994).
13. Staessen Roels H, Fagard R. Lead exposure and conventional and ambulatory blood pressure. JAMA 275:1563–1570 (1996).
14. deCastro FJ, Medley J. Lead in bone and hypertension. Matern Child Health 1:199–200 (1997).
15. Payton M, Riggs KM, Spiro A III, Weiss ST, Hu H. Relations of bone and blood lead to cognitive function: the VA normative aging study. Neurotoxicol Teratol 20:19–27 (1998).
16. Schwartz BS, Stewart WF, Todd AC, Simon D, Links JM. Different associations of blood lead, meso 2,3-dimercaptosuccinic acid (DMSA)-chelatable lead, and tibial lead levels with blood pressure in 543 former organolead manufacturing workers. Arch Environ Health 55:85–92 (2000).
17. Kim R, Rotnisky A, Sparrow D, Weiss ST, Wager C, Hu H. A longitudinal study of low-level exposure and impairment of renal function. JAMA 275:1177–1181 (1996).
18. Muldoon SB, Cauley JA, Kuler LH, Scott J, Rohay J. Lifestyle and sociodemographic factors as determinants of blood lead levels in elderly women. Am J Epidemiol 139:599–608 (1994).
19. Koss E. Lead Exposure, Inactivity Linked to Alzheimer's. Available: http://www.cnn.com/2000/HEALTH/05/04/alzheimers.lead.reut/index.html [cited 27 June 2000].
20. Heaney RP, Yates AJ, Santora AC. Bisphosphonate effects and the bone remodelling transient. J Bone Miner Res 12:1143–1151 (1997).
21. Reid DM, Hughes RA, Lann RFJM, Sacco-Gibson NA, Wenderoth DH, Adamai S, Eusebio RA, Devogelaer J-P. Efficacy and safety of daily risedronate in the treatment of corticosteroid-induced osteoporosis in men and women: a randomized trial. J Bone Miner Res 15:1006–1013 (2000).
22. Farias P, Borja-Aburto VH, Rios C, Hertz-Picciotto I, Rojas-Lopez M, Chavez-Ayala R. Blood lead levels in pregnant women of high and low socioeconomic status in Mexico City. Environ Health Perspect 104:1070–1074 (1996).
23. Hernandez-Avila M, Gonzalez-Cossio T, Palazuelos E, Romieu I, Aro A, Fishbein E, Peterson KE, Hu H. Dietary and environmental determinants of blood and bone lead levels in lactating postpartum women living in Mexico City. Environ Health Perspect 104:1076–1082 (1996).
24. Kalwarf HJ, Specker BL, Bianchi DC, Ranz J, Ho M. The effect of calcium supplementation on bone density during lactation and after weaning. N Engl J Med 337:523–528 (1997).
25. Chestnut CH, Bell NH, Clark GS, Drinkwater BL, English SC, Johnston CC Jr, Notelovitz M, Rosen C, Cain DF, Flessland KA, et al. Hormone replacement therapy in postmenopausal women: urinary N-telopeptide of type I collagen monitors therapeutic effect and predicts response of bone mineral density. Am J Med 102:29–37 (1997).
26. Liberman UA, Weiss SR, Broll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs RW Jr, Dequeker J, Favus M, et al. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. N Engl J Med 333:1437–1443 (1995).
27. Webber CE, Chettle DR, Bowins RJ, Beaumont LF, Gordon GL, Song X, Blake JM, McNutt RH. Hormone replacement therapy may reduce the return of endogenous lead from bone to the circulation. Environ Health Perspect 103:1150–1153 (1995).
28. Osteoporosis and Fractures in Men. PA-97–009. NIH Guide 25(39): (1996). Available: http://grants1.nih.gov/grants/guide/pa-files/PA-97-009.html [cited 17 July 2002].
29. Gulson BL, Mahaffey KR, Mizon KJ, Korsch MJ, Cameron MA, Vimpani G. Contribution of tissue lead to blood lead in adult female subjects based on stable lead isotope methods. J Lab Clin Med 125:703–712 (1995).
30. Manton WI. Sources of lead in blood: Identification by stable isotopes. Arch Environ Health 32:149–159 (1977).
31. Manton WI. Total contribution of airborne lead to blood lead. Br J Ind Med 42:168–172 (1985).
32. Kim R, Landrigan C, Mossmann P, Sparrow D, Hu H. Age and secular trends in bone lead levels in middle-aged and elderly men: three-year longitudinal follow-up in the normative aging study. Am J Epidem 146:586–591 (1997).
33. Baecklund M, Pederen NL, Bjorkman L, Vahter M. Variation in blood concentrations of cadmium and lead in the elderly. Environ Res 80:222–230 (1999).
34. Rabinowitz M. Toxicokinetics of bone lead. Environ Health Perspect 91:33–37 (1991).
35. Gulson BL, Gillings BR, Jameson CW. Stable lead isotopes in teeth as indicators of past domicile—a potential new tool in forensic science. J Forensic Sci 42:787–791 (1997).
36. CDC. Preventing Lead Poisoning in Young Children: A Statement by the Centers for Disease Control—October 1991. Atlanta, GA:Centers for Disease Control, 1991.
37. Gulson BL, Mahaffey KR, Jameson CW, Mizon KJ, Patison N, Smith AM, Law AJ, Korsch MJ. Dietary lead intakes for mother/child pairs and relevance to pharmacokinetic models. Environ Health Perspect 105:1334–1342 (1997).
38. Gulson BL, Mizon KJ, Palmer JM, Korsch MJ, Taylor AJ. Calcium supplementation minimizes mobilization of lead from the maternal skeleton during pregnancy and lactation—preliminary results [Abstract]. Presented at the Society of Toxicology Annual Meeting, 25–29 March 2001, San Francisco, CA.
39. Smith DR, Osterloh JD, Flegal AR. Use of endogenous stable lead isotopes to determine the release of lead from the skeleton. Environ Health Perspect 104:60–66 (1996).
40. Blake KCH, Mann M. Effect of calcium and phosphorus on the gastrointestinal absorption of ^{203}Pb in man. Environ Res 30:188–194 (1983).
41. Gulson BL, Mizon KJ, Palmer JM, Korsch MJ, Taylor AJ. Contribution of lead from calcium supplements to blood lead. Environ Health Perspect 109:283–288 (2001).
42. Heaney RP, Recker RR, Stegman MR, Moy AJ. Calcium absorption in women: relationships to calcium intake, estrogen status, and age. J Bone Miner Res 4:469–475 (1989).
43. Leggett RW. An age-specific kinetic model of lead metabolism in humans. Environ Health Perspect 101:598–616 (1993).
44. O'Flaherty EJ. Physiologically based models for bone-seeking elements IV. Kinetics of lead disposition in humans. Toxicol Appl Pharmacol 118:16–29 (1993).
45. Heaney RP. The bone-remodelling transient: implications for the interpretation of clinical studies of bone mass change. J Bone Miner Res 9:1515–1523 (1994).