Measurement Variability Associated with KXRF Bone Lead Measurement in Young Adults

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In vivo bone lead measurement using K X-ray fluorescence (KXRF) has been used to estimate long-term lead exposure, especially in adults. Relatively few studies have been conducted on young subjects with this technique. To explore the measurement variability of KXRF bone lead measurements in young subjects, the tibiae of two male cadavers from Boston, Massachusetts, 17 and 20 years of age, were obtained for repeated bone lead measurements. Bone lead concentrations were measured using a grid of nine locations, 1 cm apart, centered at the midpoint of the tibia. Each location was sampled using five 60-min measurements. Measured concentrations ranged from < 0 to 11.8 μg Pb/g bone mineral across a tibia with mean concentrations for the midpoint locations of 0.8 μg Pb/g bone mineral SD = 2.5 and 2.0 μg Pb/g bone mineral (SD = 1.9) for the left and right legs of the younger subject and 3.6 μg Pb/g bone mineral (SD = 2.6) and 6.0 μg Pb/g bone mineral (SD = 3.3) for the left and right legs of the older subject. Although bone lead concentrations did not vary significantly by measurement location in an individual leg, reported measurement uncertainty increased significantly at locations that were 1 cm from the center of the tibia horizontally ($p < 0.0001$). Symmetry in bone lead concentration between legs was observed for the 17-year-old subject. Potential asymmetry between the left and right legs was suggested for the 20-year-old subject ($p = 0.06$). These data describe the degree of variability that may be associated with bone lead measurements of young subjects with low bone lead concentrations using a standard spot-source KXRF instrument. Because of the importance of conducting additional research on adolescent lead toxicity, further improvements to the precision of KXRF measurement are needed. *Key words*: bone, cadavers, K X-ray fluorescence (KXRF), lead, young adults. *Environ Health Perspect* 108:239–242 (2000). [Online 7 February 2000]
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Environmental lead exposure continues to be pervasive around the world. As environmental exposure levels drop, new and more sophisticated techniques are necessary to measure lead exposure. Bone lead level is a measure of cumulative lead exposure because bone is the long-term repository for lead in the body and approximately 95% of the lead in adults is stored in bone (*1,2*). *In vivo* bone lead measurement using K X-ray fluorescence (KXRF) allows noninvasive measurement of bone lead levels. KXRF has been used in epidemiologic studies of both occupationally and environmentally exposed adults on three continents to identify lead-related health effects in adults (*3*).

For bone lead studies of young adults and teenagers, efforts have been made to improve the sensitivity of the instrumentation (*4*) and the measurement methodology (*5*). Measurable bone lead levels have been detected in young adults and adolescents (*5–10*). However, these studies are characterized by a relatively high measurement error to bone lead concentration ratio.

Although KXRF is a useful tool for evaluating lead-related health effects in adults, there are concerns about the use of this technology in young adults and children because of measurement limitations (*11,12*). In addition to concerns about the low levels of bone lead in young populations, the kinetics of bone growth suggest that on the cellular level, bone growth may be nonuniform, resulting in heterogeneous distribution of lead throughout the bone matrix as osteoblasts build bone (*13–15*). To explore the extent of measurement variability associated with KXRF bone lead measurement in young environmentally exposed subjects, we obtained tibia from two teenaged cadavers to perform multiple measurements using KXRF.

Materials and Methods

Four cadaveric tibiae from two teenage males were obtained from a hospital specimen bank for multiple measurements of bone lead concentration. The tibiae, collected for bone transplantation, had no overlying tissue and had been preserved by freezing. The donors were 17 years of age (subject A) and 20 years of age (subject B). They had died in traumatic accidents unrelated to lead exposure. Although no information was available regarding the subjects' medical histories, we believe that they represent normal lead exposure in the Boston, Massachusetts, area. Tibiae were full length (approximately 36 cm in length) and 3 cm in width.

Bone lead was measured using the KXRF bone lead scanner designed by our research team for low-level lead measurement. This instrument has the measurement sensitivity necessary to measure adult bone lead at low levels (*4*). The details of bone lead measurement are described elsewhere (*4,5*). Briefly, the instrument used a ^{109}Cd γ-ray source of activity 1.11 GBq and a high-purity germanium detector in a back-scatter geometry. The source to bone distance was 2 cm. The collimator was positioned perpendicular to the anterior tibial surface and the distance between the source and bone was measured by the technician. X-ray and γ-ray signals were shaped and digitized and then acquired by a multichannel analyzer board in a personal computer. The lead X-rays were normalized to the elastic scatter peak; the elastic scatter peak was primarily due to elements of bone mineral (*16*). Normalization rendered the accuracy of measurement relatively insensitive to variations of bone shape, size, and density. A 60-min sampling time was used to compare the results to those from previous studies of young adults (*5,6,9*). The bone lead scanner reported two values for each measurement: a weighted mean estimate of the lead concentration based on the $K_{\alpha 1}$ and $K_{\beta 1}$ peaks and an estimate of measurement uncertainty based on the counting statistics and fitting algorithms. The measurement uncertainty was derived by a goodness-of-fit calculation of the scatter in the KXRF spectrum and represented an estimate of the SD of multiple measurements.

Five 1-hr measurements were made at nine locations on each tibia. Locations near the midpoint were sampled to evaluate bone heterogeneity. A template centered at the midpoint of the tibia was used to mark the locations 1 cm above and below the midpoint and 1 cm to the right and left of the

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center. Sample locations were marked directly on the bone with a lead-free indelible marker. Figure 1 details the measurement locations. All measurements from one location were collected over one 5-hr period to reduce measurement variability associated with sample handling. The tibia was restrained with straps to prevent shifting during sample collection. Tibiae were stored in a freezer between measurements to prevent biologic growth on bone tissue. All data were collected over a 1-month period.

We used the STATA program (*17*) for descriptive statistics and graphical display of the data. Analysis of variance (ANOVA) techniques were used to evaluate the role of location on bone lead concentration and measurement uncertainty. Student's *t*-tests were used to test differences between subjects and between legs.

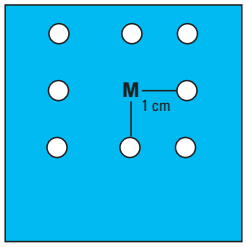

Figure 1. Measurement locations on shaft of the tibia. All locations, except the midpoint of the tibia (M), are 1 cm from the tibia midpoint.

Results

Bone lead concentration was measured 5 times at nine locations on each tibia. One hundred seventy-seven of 180 possible measurements were completed on these four tibia. In three instances, the scanner failed to complete the fifth measurement at that location because of a data storage error. Point estimates of bone lead concentration ranged from - 2.4 to + 9.6 µg Pb/g bone mineral (µg/g) for subject A and from - 3.7 to + 11.8 µg/g for subject B. Figure 2 shows lead concentrations at each measurement location as box plots for subjects A and B. Summary statistics for each tibia and midpoint are presented in Table 1. The overall mean bone lead concentration was significantly greater for subject B (subject A: overall mean = 2.6, SD = 2.6; subject B: overall mean = 4.5, SD = 3.1, $p < 0.0001$). The coefficients of variation for measures from the tibia midpoint ranged from 55% for the right leg of subject B to 320% for the right leg of subject A.

When data from both subjects were analyzed together, there was no significant difference in values for the right and left legs of the subjects using two-way ANOVA to control for measurement location and subject. When restricted to subject B, there was a marginally significant difference between the left and right leg lead levels ($p = 0.06$).

Overall, lead concentration did not differ by measurement location. Again, when restricted to subject B, a marginally significant difference among the nine leg locations was observed using ANOVA ($p = 0.1$). However, as illustrated in Figure 2, the range of observations at each location was broad in both subjects.

Measurement uncertainty associated with bone lead measurements was influenced by horizontal location; measurements from the center of the tibia had significantly lower measurement uncertainty than measurements from either the right or left of center ($p < 0.0001$) (Figure 3). No differences were observed for vertical changes from the midpoint. Uncertainty estimates by subject and leg are presented in Table 2.

In ANOVA models, the SD for the mean bone lead concentration at each location did not differ from the average reported measurement uncertainty for that location. The ratio of the actual SD to the reported measurement uncertainty was 1.03 for subject B and 1.10 for subject A.

Discussion

Using the bone lead scanner that we used in previous studies, we measured the lead concentration at nine locations near the midpoint of the tibia in both legs of two male cadavers 17 and 20 years of age. Bone lead concentrations differed between subjects and marginally between legs for one subject. Bone lead concentrations were variable, although not statistically different, by measurement location on the tibia. Reported measurement uncertainty was significantly higher at locations that were 1 cm from the center of the tibia horizontally (Figure 3).

The bone lead levels observed in these two subjects are consistent with other low values measured for young adult subjects from the Boston area (*5–7*). However, as shown in Figure 4, the variability in the measures from the tibia midpoint overlap with the population distributions observed in these other studies. Figure 4 suggests that

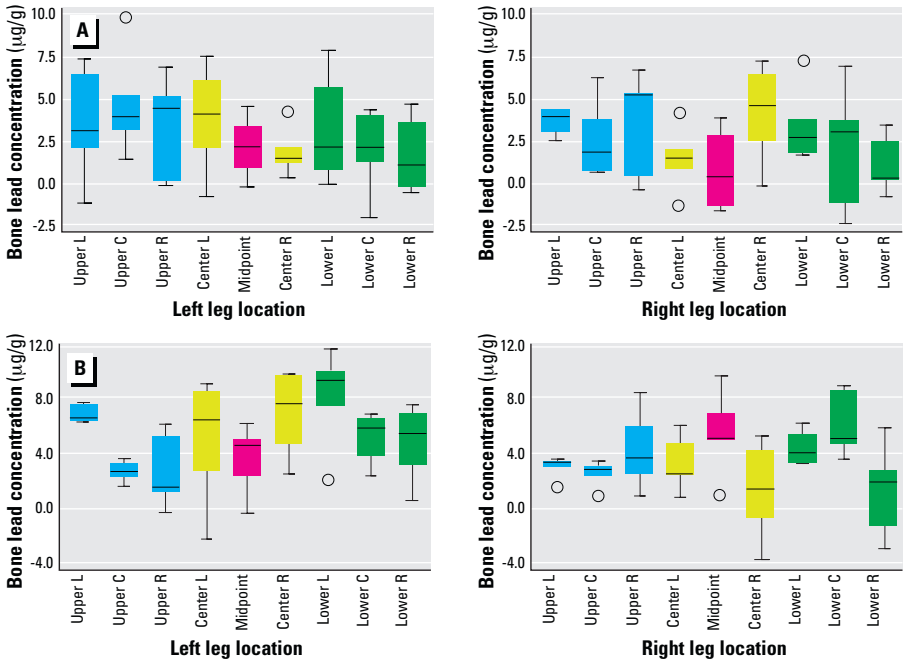

Figure 2. Box plots of bone lead measurements by subject and leg. Shown are the median (center line in box); the 25th and 75th percentiles (the top and bottom borders of the box), 3/2 the interquartile range (the vertical lines), and the extreme values (circles) (*17*). Location codes: upper, 1 cm above midpoint; center, at midpoint vertically; lower, 1 cm below midpoint; L, 1 cm left of center; C, at midpoint horizontally; and R, 1 cm right of center. (*A*) Bone lead measurements for subject A (17-year-old male, Boston, MA, 1995). (*B*) Bone lead measurements for subject B (20-year-old male, Boston, MA, 1995).

Table 1. Summary by subject and leg of bone lead levels measured by KXRF.

Location	Measurements	Bone mineral (µg Pb/g) Mean ± SD[a]	Range
Subject A[b]			
Left leg[c]	44	2.7 ± 2.7	-2.3–9.6
Midpoint[d]	4	2.0 ± 1.9	-0.4–4.3
Right leg[c]	45	2.6 ± 2.7	-2.4–8.0
Midpoint[d]	5	0.8 ± 2.5	-1.7–3.8
Subject B[e]			
Left leg[c]	44	5.2 ± 3.2	-2.4–11.8
Midpoint[d]	5	3.6 ± 2.6	-0.4–6.2
Right leg[c]	44	3.9 ± 3.0	-3.7–10.3
Midpoint[d]	5	6.0 ± 3.3	1.3–10.3

[a]Mean Pb concentration. [b]17-year-old male. [c]Represents the average of all measurements at the nine locations. [d]Midpoint values are included in the summary results for each leg. [e]20-year-old male.

a major portion of the observed population variability in young subjects is associated with variability in the bone lead measurement.

The distribution of lead in the bones of young adults and teenagers has not been well characterized. Lead homogeneity has been investigated in the bones of older adults using both destructive and nondestructive methods (*18–21*). Wittmers et al. (*19*) used atomic absorption analysis and found no significant difference in the cortical bone lead concentrations of adult cadavers along the length of the tibia shaft, although some variation in lead concentration was reported. Schidlovsky et al. (*20*) analyzed cortical bone samples from adults with proton-induced X-ray emission (PIXE), a technique with the ability to sample a much smaller area than atomic absorption spectroscopy or KXRF, and found that the variation in bone lead in the tibia occurred on the microscale measured by PIXE rather than the macroscale measured by KXRF. Lindh et al. (*18*) reported that the distribution of lead in the femur of a lead-exposed worker was not uniform, whereas the distribution of lead in the femur was uniform in an environmentally exposed adult. Our data are suggestive of differences in bone lead concentration by location (Figure 2B); however, we have insufficient power in our overall ANOVA analysis to demonstrate statistically significant differences between locations. Whether this difference represents actual heterogeneity or the result of measurement variability at low levels cannot be determined by KXRF analyses. In adults, symmetry between the left and right sides of the body and along the tibia shaft have been demonstrated using both cadavers and living subjects (*19,21*). In one subject, we detected a marginally significant difference in mean bone lead concentration between the left and right legs.

The measurement uncertainty has been described as an estimate of the SD of multiple measurements (*4,21*). In experiments with standards of known concentration, the reported measurement uncertainty underestimated the actual SD of multiple measurements by up to 30% (*21*). *In vivo* measurements from adults suggest that the reported measurement uncertainty underestimates the actual SD by 18% (*21*). Uncertainty estimates for our bone samples were not significantly different from the actual SD; the reported values were 3–10% lower than the actual values. The good agreement between the reported and actual SD in our study may be associated with the absence of overlying tissue on the tibiae, the consistent position for all five measurements, and the 60-min measurement time.

Even with the high relative measurement error, significant associations have been seen with bone lead levels in young adults. A study of 23 subjects 18–20 years of age measured for two 60-min periods found a strong correlation of bone lead with age (*5*). In a study of 10- to 13-year-olds, Needleman et al. (*8*) found an increased rate of delinquency in subjects with higher bone lead levels. Kim et al. (*22*) found that dentine lead levels at 6 years of age were associated with bone lead levels in young adults 18–22 years of age. In a Mexico City population of 11- to 21-year-olds, Farias et al. (*9*) demonstrated significant correlations with environmental lead sources and bone lead concentration.

Other investigations have had difficulty identifying differences in bone lead levels among subjects younger than 21 years of age. In a large study using this KXRF instrument, no associations were identified between bone lead levels and demographic and environmental factors in 167 subjects who were 13–19 years of age, although the bone lead levels measured were comparable to those seen in previous studies (*6*). In a community based study of environmentally exposed subjects, the bone lead levels of the subjects younger than 20 years of age were not statistically different from zero (*23*). In a cohort of subjects with known childhood lead exposure, bone lead levels measured at 19–21 years of age were not significantly different between exposed and unexposed subjects;

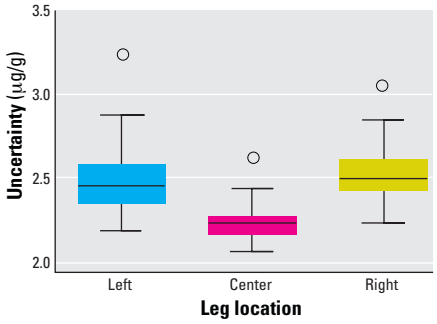

Figure 3. Box plots of measurement uncertainty by location across tibia shaft. Summary results for both subjects and both legs. Shown are the median (center line in box); the 25th and 75th percentiles (the top and bottom borders of the box), 3/2 the interquartile range (the vertical lines), and the extreme values (circles) (*17*). Left = 1 cm left of the center line of the tibia; center = midpoint of the tibia + samples 1 cm above and below; right = 1 cm right of the center line of the tibia.

Table 2. Summary by subject and leg of reported measurement uncertainty for bone lead levels measured by KXRF.

Location	Measurements	Bone mineral (µg Pb/g) Mean ± SD[a]	Range
Subject A[b]			
Left leg[c]	44	2.4 ± 0.2	2.1–3.2
Midpoint[d]	4	2.2 ± 0.1	2.1–2.3
Right leg[c]	45	2.4 ± 0.2	2.1–2.8
Midpoint[d]	5	2.2 ± 0.1	2.1–2.3
Subject B[e]			
Left leg[c]	44	2.4 ± 0.2	2.1–2.9
Midpoint[d]	5	2.2 ± 0.1	2.1–2.3
Right leg[c]	44	2.4 ± 0.2	2.1–3.0
Midpoint[d]	5	2.3 ± 0.1	2.3–2.4

[a]Mean measurement uncertainty. [b]17-year-old male. [c]Represents the average of all measurements at the nine locations. [d]Midpoint values are included in the summary results for each leg. [e]20-year-old male.

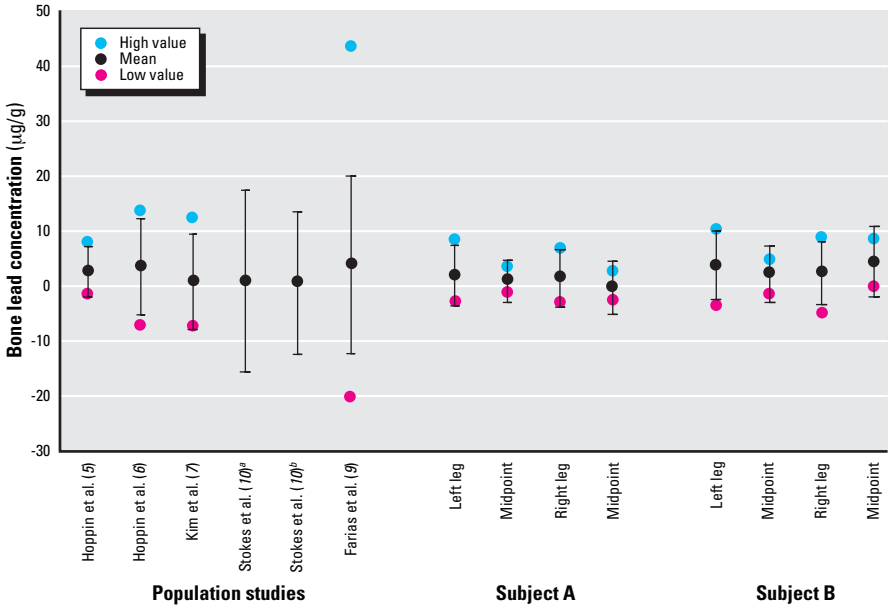

Figure 4. Comparison of bone lead concentrations measured in subjects 14–21 years of age using KXRF. Studies include Hoppin et al. (*5*) (n = 23, age 18–21 years, 2 × 60-min measurements); Hoppin et al. (*6*) (n = 168, age 13.5–19 years, 1 × 60-min measurement); Kim et al. (*7*) (n = 58, age 18.7–21.8 years, 1 × 30-min measurement); Stokes et al. (*10*) (exposed, n = 58, age 19–21 years, 1 × 30-min measurement; unexposed, n = 61, age 19–21 years, 1 × 30-min measurement); Farias et al. (*9*) (n = 98, age 11–21 years, 1 × 60-min measurement). [a]Exposed. [b]Unexposed.

however, a significant elevation in bone lead concentration was noted among exposed subjects older than 21 years of age (*10*). The inability to detect differences in bone lead concentrations in this age group using KXRF may be associated with a variety of issues, including the relatively high measurement error in young subjects, the heterogeneity of the bone matrix with respect to lead in the growing subjects, a true lack of difference in bone lead levels, or some combination of all of these. A Canadian study of bone lead concentration in cadaver vertebrae found the lowest bone lead concentrations among subjects 12–19 years of age, with significantly higher concentrations among both younger (1–11 years of age) and older subjects (> 20 years of age) (*24*). Thus, the 12- to 19-year age range may have the lowest bone lead concentration and therefore may be the most difficult to study with KXRF.

Measuring low-level lead exposure in teenagers and young adults is challenging. Even with a relatively sensitive bone lead scanner that provides an accurate estimate of the variability in the reported bone lead concentration, the potential for bone heterogeneity and measurement variability limit the interpretations of bone lead concentrations reported with one measurement. In our study, heterogeneity was observed to some extent both between legs and among locations. The impact of potential heterogeneity of the bone matrix can not be adequately evaluated in light of the low lead concentrations present and the inherent measurement variability of the KXRF method. Further exploration of this issue should utilize atomic absorption spectrometry or PIXE to identify patterns of spatial variation of lead in the bones of young adults. Nevertheless, even with the high degree of measurement error relative to lead concentration, investigators continue to see subtle effects of bone lead levels in young adult subjects, especially those with known lead exposure who are older than 20 years of age. Because of the importance of understanding the relationship of lead exposure to behavioral outcomes in teenagers (*8*), additional research should be conducted to improve the precision of KXRF measurements in this age group.

References and Notes

1. Barry PSI. A comparison of concentration of lead in human tissues. Br J Ind Med 32:119–139 (1975).
2. Rabinowitz MB. Toxicokinetics of bone lead. Environ Health Perspect 91:33–37 (1991).
3. Hu H. Bone lead as a new biologic marker of lead dose: recent findings and implications for public health. Environ Health Perspect 106(suppl 4):961–967 (1998).
4. Aro ACA, Todd AC, Amarasiriwardena C, Hu H. Improvements in the calibration of ^{109}Cd K X-ray fluorescence systems for measuring bone lead *in vivo*. Phys Med Biol 39:2263–2271 (1994).
5. Hoppin JA, Aro ACA, Williams PL, Ryan PB, Hu H. Validation of bone lead measurement in young adults. Environ Health Perspect 103:78–83 (1995).
6. Hoppin JA, Aro ACA, Ryan PB, Hu H. *In vivo* bone lead measurement in suburban teenagers. Pediatrics 100:365–370 (1997).
7. Kim R, Hu H, Rotnitzky A, Bellinger D, Needleman H. A longitudinal study of chronic lead exposure and physical growth in Boston children. Environ Health Perspect 103:952–957 (1995).
8. Needleman HL, Riess JA, Tobin MJ, Biesecker GE, Greenhouse JB. Bone lead levels and delinquent behavior. JAMA 275:363–369 (1996).
9. Farias P, Hu H, Rubenstein E, Meneses-Gonzalez F, Fishbein E, Palazuelos E, Aro A, Hernandez-Avila M. Determinants of bone and blood lead levels among teenagers living in urban areas with high lead exposure. Environ Health Perspect 106:733–737 (1998).
10. Stokes L, Letz R, Gerr F, Kolczak M, McNeill FE, Chettle DR, Kaye WE. Neurotoxicity in young adults 20 years after childhood exposure to lead: the Bunker Hill experience. Occup Environ Med 55:507–516 (1998).
11. National Academy of Sciences, National Research Council. Measuring Lead Exposure in Infants, Children, and Other Sensitive Populations. Washington, DC: National Academy Press, 1993.
12. Rosen JF, Pounds JG. Severe chronic lead insult that maintains body burdens of lead related to those in the skeleton: observations by Dr. Clair Patterson conclusively demonstrated. Environ Res 78(2):140–151 (1998).
13. Bryant FJ, Loutit JF. The entry of strontium-90 into human bone. Proc R Soc London Ser B 159:449–465 (1964).
14. Hui SL, Johnston CC Jr, Mazess RB. Bone mass in normal children and young adults. Growth 49:34–43 (1985).
15. O'Flaherty EJ. Physiologically-based models for bone-seeking elements. V: Lead absorption and disposition in childhood. Toxicol Appl Pharmacol 131:297–308 (1995).
16. Chettle DR, Scott MC, Somervaille LJ. Lead in bone: sampling and quantitation using K X-rays excited by ^{109}Cd. Environ Health Perspect 91:49–55 (1991).
17. Stata Corporation. Stata Reference Manual: Release 3.1. 6th ed. College Station, TX:Stata Corporation, 1993.
18. Lindh U, Brune D, Nordberg G. Microprobe analysis of lead in human femur by proton induced X-ray emission (PIXE). Sci Total Environ 10:31–37 (1978).
19. Wittmers LE, Wallgren J, Alich A, Aufderheide AC, Rapp G. Lead in bone. IV: distribution of lead in the human skeleton. Arch Environ Health 43:381–391 (1988).
20. Schidlovsky G, Jones KW, Burger DE, Milder FL, Hu H. Distribution of lead in human bone. II: Proton microprobe measurements. Basic Life Sci 55:275–280 (1990).
21. Gordon CL, Webber CE, Chettle DR. The reproducibility of ^{109}Cd-based X-ray fluorescence measurements of bone lead. Environment Health Perspect 102:690–694 (1994).
22. Kim R, Hu H, Rotnitzky A, Bellinger D, Needleman H. Longitudinal relationship between dentin lead levels in childhood and bone lead levels in young adulthood. Arch Environ Health 51:375–382 (1996).
23. Kosnett MJ, Becker CE, Osterloh JD, Kelly TJ, Pasta DJ. Factors influencing bone lead concentration in a suburban community assessed by noninvasive K X-ray fluorescence. JAMA 271:197–203 (1994).
24. Samuels ER, Meranger JC, Tracy BL, Subramanian KS. Lead concentrations in human bones from the Canadian population. Sci Total Environ 89:261–269 (1989).