Age and Secular Trends in Bone Lead Levels in Middle-aged and Elderly Men: Three-Year Longitudinal Follow-up in the Normative Aging Study

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The goal of this study was to examine age and secular trends in bone and blood lead levels of community-exposed men. Bone and blood lead levels were measured twice, with a 3-year interval between measurements, among participants in a longitudinal study of aging. Seventy subjects (mean age = 66 years, age range = 52–83 years) with repeated measurements of bone and blood lead levels were studied. At the first evaluation, geometric mean lead levels in patella, tibia, and blood were 29.1 (standard deviation (SD) 1.8) /g/g, 17.5 (SD 2.0) /g/g, and 6.7 (SD 1.8) /g/dl, respectively. At the second evaluation, these levels were 22.2 (SD 1.8) /g/g, 17.9 (SD 1.7) /g/g, and 5.1 (SD 1.4) /g/dl, respectively. Cross-sectional analysis of each set of measurements indicated that, on average, a 1-year-older individual would have 2.7% and 2.4–3.2% higher levels of lead in patella and tibia, respectively. In contrast to the increasing age trend in cross-sectional analysis, the secular trend over time was decreasing for patella lead levels and stable for tibia lead levels. The authors conclude that in community-exposed middle-aged and elderly men, the biomarkers of exposure to lead are decreasing in patella and blood and unchanging in tibia as of the early 1990s. The increasing age trend in bone lead levels in cross-sectional studies should be carefully interpreted in light of the birth cohort effect. Am J Epidemiol 1997;146:586–91.

Because the skeleton stores 90–95 percent of an adult’s lead burden, the levels of lead in bone serve as an index of cumulative exposure to lead over years to decades (1–3). Several studies have reported that bone lead levels have a strong positive association with age (4–10). However, these cross-sectional studies did not clearly determine whether this association is due to a continuous increase with aging in the amount of lead stored in bones or whether it simply reflects a generation effect arising from different levels of cumulative lead exposure between younger and older individuals, i.e., a birth cohort effect of exposure.

The implementation of strict environmental controls has resulted in a negative balance between the absorption and the excretion of environmental lead by the general population in the United States (11). According to a recent report based on the National Health and Nutrition Examination Surveys II and III (12), mean blood lead levels have decreased by 72–77 percent over the last two decades.

Longitudinal studies of occupationally exposed individuals (13, 14) suggest that bone lead levels decrease after occupational exposure ends. Few studies have specifically examined secular trends in the total body burden of lead in the general population in the 1990s. We report a longitudinal study of age and secular trends in bone lead levels in a cohort of community-exposed men from the Normative Aging Study (15).

MATERIALS AND METHODS

Study subjects

The Normative Aging Study is a multidisciplinary longitudinal study of aging established by the Veterans Administration in 1961, when 2,280 community-dwelling men from the Greater Boston area (21–80 years of age) were enrolled. At the outset, candidates for participation were screened by health criteria, and those free of known chronic medical conditions were enrolled (16). Since 1991, we have been measuring bone and blood lead levels in Normative Aging Study participants. For the current study, we selected the...
individuals with two measurements of bone and blood lead levels separated by a 3-year interval. The study protocol was approved by the institutional review boards of the Department of Veterans Affairs Outpatient Clinic and the Brigham and Women's Hospital (both in Boston, Massachusetts). Written, informed consent was obtained from all participants.

**Measurement of bone lead levels**

Lead levels in the tibia and the patella were measured in vivo with a K x-ray fluorescence instrument (ABIOMED, Inc., Danvers, Massachusetts). The physical principles, technical specifications, validation, and quality control procedures of this (17, 18) and other K x-ray fluorescence instruments (19, 20) have been described in detail elsewhere. In short, this instrument uses a $^{109}$Cd gamma ray source to provoke the emission of fluorescent photons from target tissue; the photons are then detected, counted, and arrayed on a spectrum (21). The net lead signal is determined by a linear least-squares algorithm after subtraction of Compton background counts. The lead fluorescence signal is then normalized to the elastic or coherently scattered x-ray signal, which arises predominantly from the calcium and phosphorus present in bone mineral. The unit of measure so derived is the number of micrograms of lead per gram of bone mineral.

For the present study, 30-minute measurements were made at the midshaft of the left tibia and at the left patella after each region had been washed with a 50 percent solution of isopropyl alcohol. The K x-ray fluorescence beam collimator was set perpendicular to the bone surface for the tibia and at 30° in the lateral direction for the patella.

**Measurement of blood lead levels**

Blood was collected in a special lead-free tube containing ethylenediaminetetraacetic acid and was sent to ESA Laboratories, Inc. (Bedford, Massachusetts), for lead determination. In the laboratory, blood samples underwent digestion with nitric acid at room temperature. The resulting solution was centrifuged, and the supernatant was poured into a sample cup and analyzed by Zeeman background-corrected flameless atomic absorption spectrometry. The instrument was calibrated with blood lead standard materials from the National Bureau of Standards, and the calibration was rechecked after the testing of every 21 samples. Twenty-four measurements by this method gave a mean of 5.3 (standard deviation 1.2) mg/dl; the target of the National Bureau of Standards is 5.7 mg/dl.

**Statistical analysis**

Log transformation of lead biomarker values improved the normality of distribution and resulted in a slightly stronger correlation with age. We added 1 to the values of lead biomarkers before log transformation to set the zero point for the transformed values to be the same as that for nontransformed values. For quicker interpretation, we mostly reported results with values reexpressed in original units.

We evaluated the secular trend in lead biomarker levels using paired $t$ tests. To check whether this trend resulted from spurious data for a particular subgroup, we divided the subjects into seven subgroups of 10 subjects each according to initial lead level, and we compared the mean levels of bone lead on the nontransformed scale at the first and second measurements.

We assessed the age trend at each measurement and then examined the consistency of the cross-sectional age effect and the age-matched time trend (22). For this purpose, we examined the graphs of locally weighted scatterplot smoothing (lowess) (23) of cross-sectional data on age and bone lead at baseline and at the time of follow-up measurements.

If both age and secular trends show similar patterns, the apparent positive association between age and bone lead levels previously reported (4–10) may be the result of continuous accumulation of lead in the skeletons of the aging individuals and population. However, if age and secular trends differ (e.g., increasing age trends but not increasing secular trends), the apparent age effect in cross-sectional studies may be merely the result of a birth cohort effect that is due to secular changes in levels of lead exposure over calendar time.

Because the values for dependent variables in the linear regression were on the logarithmic scale, the cross-sectional age trend was algebraically assumed to be exponential. Therefore, we present the predicted percent change in lead levels corresponding to a 1-year age difference.

**RESULTS**

A total of 70 subjects underwent two measurements of bone and blood lead levels from August 1991 through April 1995. The study subjects were 52–83 years of age (mean, 65.7 years) at the first measurement in 1991–1992 (table 1). The mean interval between the first and second bone lead measurements was 3.0 (standard deviation 0.3) years.

Over 3 years, geometric mean lead levels in patella decreased by 23 percent (95 percent confidence interval (CI) 14–31 percent), which is equivalent—on the

<table>
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* SD, standard deviation.† The geometric mean of the second measurement was significantly lower than that of the first measurement (p < 0.001).

nontransformed scale—to 6.9 µg/g (95 percent CI 4.3–9.2 µg/g). In contrast, geometric mean tibia lead levels did not change significantly over this interval.

Patella lead levels decreased in all seven subgroups except that with the lowest initial levels, and tibia lead levels decreased in the top four subgroups (figure 1). In general, a subgroup with initial mean bone lead levels >20 µg/g had lower mean levels 3 years later.

In cross-sectional analyses, bone lead levels were positively associated with age at both examinations. Smoothing curves representing the age trends in patella or tibia lead levels at both examinations were overlaid at each graph in figure 2, respectively. The slopes of two smoothing curves were similar for both patella and tibia lead levels, suggesting that the pattern of cross-sectional age trends is invariable over time.

The vertical distance between the curves at a given age point in each graph represents the age-matched secular trend in mean bone lead levels (22). The downward shift of the smoothing plot for patella lead levels indicates a decreasing secular trend in age-adjusted patella lead levels over 3 years in calendar time. For tibia lead levels, the shift was close to zero, indicating an unchanging secular trend. The difference between the patterns of secular and age trends suggested the importance of accounting for secular trends to avoid possible misinterpretation of the age trends.

Regression analysis of two sets of cross-sectional data (table 2) predicted that, on average, a 1-year older individual would have approximately 2.7 percent and 2.4–3.2 percent higher levels of lead in patella and tibia, respectively. For subjects with bone lead levels around the geometric mean values, being 1 year older (i.e., being born 1 year earlier) than others at one point in time was associated with patella and tibia lead levels 0.60 µg/g and 0.56–0.74 µg/g higher, respectively.

DISCUSSION

In this 3-year follow-up study of a sample of elderly men, we found that age and secular trends in bone lead levels were not the same, especially for patella lead. The age- and time-related changes in patella lead levels predicted by a 3-year increase in age and in calendar time were an 8 percent increase and a 23 percent decrease, respectively. For tibia lead levels, the predicted changes were a 10 percent increase and no change, respectively.

Studies based on autopsy and biopsy material (1-3) as well as in vivo measurements of lead in bone
(4-10) have suggested that bone lead levels in the general population increase with age. Most studies have related bone lead levels to a linear (6, 8-10), exponential (5), or quadratic (7) function of age.

In 1991, Kosnett et al. (8) surveyed 100 suburban residents and found that age was the strongest predictor of tibia lead levels, which were measured by the same K x-ray fluorescence technique used in our study. The slope in their study was 0.38 ppm/year, on average, for individuals between the ages of 20 and 55 years, with an additional upward inflection for men older than 55 years. More recently, Hu et al. (10) examined the determinants of bone lead levels in 719 subjects in the Normative Aging Study. The slopes of age for patella and tibia lead levels in multiple regressions, with adjustment for smoking and education, were increases of 0.41 μg/g (95 percent CI 0.15-0.66 μg/g) and 0.63 μg/g (95 percent CI 0.51-0.76 μg/g), respectively, per 1 year of age. The cross-sectional age trends in patella and tibia lead levels observed in our study—0.60 μg/g and 0.56-0.74 μg/g per 1 year of age, respectively—are quite consistent with those reported by both Kosnett et al. (8) and Hu et al. (10).

The level of environmental exposure to lead in the general population of most developed countries has declined remarkably since the 1970s (24, 25). Drasch et al. (4) first suggested that the body lead burden of the population might be reduced in an area where environmental exposure to lead was declining. Determining the concentrations of lead in the bones of non-occupationally exposed adults who died in southern Bavaria (Germany) in 1984-1985, the investigators compared these levels with those measured in 1974 by comparable methods in comparable populations in the same area. The researchers noticed that lead levels in femoral and pelvic bones decreased over this decade in almost all age groups. According to our estimation based on their published data, geometric mean lead levels in femoral and pelvic bones of subjects 50-70 years of age decreased by 6 percent and 37 percent, respectively, during this 10-year period. The authors pointed out that the decline might have re-
resulted from the legally mandated reduction in the lead content of gasoline from 0.4 to 0.15 g/liter in Germany in 1976. They hypothesized that the total removal of lead from gasoline would reduce the body burden still further. Because the study of Drasch et al. (4) was a crude between-person comparison with a relatively small sample size (n = 27 for the baseline measurements of 1974), this inference may have been vulnerable to confounding and selection bias.

Our finding of a significant decline in patella lead levels over 3 years is compatible with the observations of Drasch et al. and strongly supports their hypothesis. Because our longitudinal observation is based on a within-person comparison nested in a well-characterized larger cohort living in an area with declining environmental exposure to lead, the inference drawn therefrom may be more valid and reliable. Furthermore, our finding of declining lead levels in the patella, a trabecular bone, is consistent with what is known about the toxicokinetics of bone lead (26). Since lead has a faster turnover rate in trabecular bones than in the cortical bones, trabecular bone lead is more sensitive to the changes in recent exposure.

If we assume that exposure levels were constant over time, we can estimate the half-life of patella lead from our data. Given that it took 3.0 years for a 23 percent reduction (95 percent CI 14–31 percent), it will take 8.0 years for a 50 percent reduction (95 percent CI 5.7–13.9 years) if the reduction is a simple exponential decrease. Because bone lead levels are in a dynamic equilibrium with lead levels in blood, and blood lead levels are decreasing continuously in the United States (12), the half-life of patella lead calculated here should not be taken as definitive.

Unlike patella lead levels, tibia lead levels did not change significantly over 3 years. However, the small sample size, the relatively short follow-up period, and measurement errors may have reduced the statistical power of our study to detect a modest change in tibia lead levels, if any.

The discordance between the age and secular trends in this study provides an example of birth cohort or generation effects in cross-sectional studies. The positive association between age and bone lead levels observed in previous cross-sectional studies (4–10) may have been subject to such effects and must be carefully interpreted. The younger generation has been exposed to lower levels of environmental lead than the older generation. This difference—as opposed to the aging process itself or continuous cumulative exposure—may account for the positive association between age and bone lead levels observed in cross-sectional studies.

One might ask whether the observation of rapid decline in patella lead levels within 3 years indicates that levels will be low in all subjects (regardless of age) within a relatively short period, the result being the disappearance of the age trend in cross-sectional studies. This might not be the case because the amount of decline in a given individual will be proportional to the prior level in that individual. Even though absolute levels of bone lead may decline across all age groups, relative levels with respect to age (reflecting historical exposure) will be preserved. Moreover, the decline apparently triggered by stricter environmental controls may be decelerated as a result of persistent, ubiquitous low-level exposure in industrialized society. It is noteworthy that the level of environmental lead exposure is still several hundred times higher for contemporary people than for preindustrial people (27).

Levels of lead in bone have been associated with impaired hematopoiesis (28), elevated blood pressure (29), and decreased cognitive performance (30). The toxicologic significance of bone lead levels is also demonstrated by the stronger correlation of lead levels in bone than of levels in whole blood with levels of lead in plasma (31), which is the most accurate biomarker of internal dose that reaches target organs.

Although the decline in bone lead levels in middle-aged and elderly men is a sign of successful environmental control in the past few decades, it also signals the urgent need for further research on the effect of declining bone lead levels on lead levels elsewhere—e.g., blood and plasma—and eventual health outcomes. This issue is especially important for elderly people, whose bones are a potential source of internal exposure to lead in the 1990s.

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