Determinants of Bone and Blood Lead Levels among Community-exposed Middle-aged to Elderly Men
The Normative Aging Study

Howard Hu,¹,² Marinelle Payton,¹ Susan Kornck,¹,² Antonio Aro,¹ David Sparrow,³ Scott T. Weiss,¹ and Andrea Rotnitzky⁴

Levels of lead in bone serve as a dosimeter for cumulative exposure to lead; moreover, lead in bone may serve as an internal source of circulating lead many years after environmental exposure has ceased. The authors measured lead in blood and used a K-x-ray fluorescence instrument to measure lead in the tibia (cortical) and patella (trabecular) bones in a cross-sectional survey of 719 middle-aged to elderly male participants in the Normative Aging Study who were without unusual occupational exposures to lead and who were healthy when enrolled in 1962–1965. Blood lead levels ranged from <1 to 27.9 µg/dl, with a geometric mean of 5.7 µg/dl. Tibia and patella lead level ranges (geometric means) were <1–51 (20.8) µg/g and 3–77 (29.8) µg/g, respectively. In backwards elimination multivariate regression models that considered age, race, education, retirement status, measures of both current and cumulative smoking, and alcohol consumption, the factors that remained significantly related to higher levels of both tibia and patella lead were higher age and measures of cumulative smoking, and lower levels of education. In the final model predicting blood lead that began with these same covariates and also included tibia and patella lead, the factor that accounted for the dominant portion of the variance in blood lead was patella lead. After adjustment for measurement error, a rise in patella lead from the median of the lowest to the median of the highest quintiles (13–56 µg/g) corresponded to a rise in blood lead of 4.3 µg/dl. The authors conclude that bone lead levels are substantial and comprise the major source of circulating lead in these men. Am J Epidemiol 1996;144:749–59.

alcohol drinking; blood; bone and bones; education; fluorescence; lead; radiography; smoking

Beginning in the 1970s, as the scientific community began to compile evidence of adverse health outcomes associated with lead, a number of federal regulatory and legislative efforts were undertaken to reduce lead hazards in the United States, including actions to limit the use of lead in paint, solder, and gasoline (1). These efforts have been successful in part, as reflected by the 72–77 percent decline in mean blood lead levels of children surveyed in the 1976 second National Health and Nutrition Examination Survey (NHANES II) and the 1991 NHANES III (2).

Despite these declines in blood lead, however, there remains strong concern regarding the lingering effects of lead that has accumulated in bone (3). Autopsy studies clearly indicate that the skeleton is the site of storage for around 95 percent of lead in the adult human body (4, 5). Toxicokinetic studies have demonstrated that the half-life of lead in bone is on the order of years to decades (6); nevertheless, bone is also a dynamic organ with respect to lead, evincing a constant low-level interchange with soft-tissue compartments.

With the development of in vivo K-x-ray fluorescence (KXRF), it is now possible to directly measure levels of lead retained in bone (7). Up until now, however, most investigations have focused on occupationally exposed groups who can be expected to have accumulated large lead burdens from their occupational activities. Few investigations have been conducted on community-exposed populations (8–13), and most of these studies have been on small samples of 100 or fewer persons.
In this paper, we report the results of a survey of bone and blood lead levels in 719 community-exposed middle-aged and elderly men and the relations of bone and blood lead levels to age, race, education, retirement, smoking, and alcohol consumption. All research reported herein was approved by the Human Research Committees of the Brigham and Women's Hospital and the Department of Veterans Affairs.

MATERIALS AND METHODS

Study subjects

Study subjects were participants of the Normative Aging Study, a longitudinal study of aging established by the Veterans Administration in 1961 (14). The study cohort consists of 2,280 community-dwelling men from the Greater Boston area who were aged 21–80 years on enrollment in the study. These men were recruited by newspaper and radio advertisements and by announcements in a number of local industries in eastern Massachusetts. Veterans as well as nonveterans were enrolled. Subjects came from a wide variety of occupations, although few, if any, were employed in primary lead industries (such as battery manufacturing and lead smelting). Volunteers were evaluated and selected for follow-up based on their current health status, past medical histories, and physical examinations (14). Volunteers who had a history of or presence of such chronic conditions as heart disease, hypertension, diabetes mellitus, cancer, peptic ulcer, gout, recurrent asthma, bronchiitis, or sinusitis were not admitted to the study. Also disqualified were men with either systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg. Biochemical parameters were not considered as eligibility criteria. Study subjects have returned for examinations every 3–5 years over the follow-up period, with an attrition rate of roughly 1 percent per year over the life of the study. At each visit, an extensive physical examination was carried out and laboratory, anthropometric, and questionnaire data were collected.

Information on smoking and alcohol ingestion has been collected since the inception of the study. At each subject's visit, smoking status was defined as never smoker, current smoker (of ≥1 cigarette/day), or former smoker (no smoking for at ≥30 days preceding the visit). For current and former smokers, lifetime number of pack-years of smoking was calculated. Each subject indicated whether or not, on average, he drank ≥2 alcoholic drinks per day.

Beginning in 1991, during the course of each continuing participant's regularly scheduled evaluation at the Department of Veterans Affairs Outpatient clinic in Boston, a fresh whole blood specimen was taken for measurement of lead and permission was sought to take KXRIF bone lead measurements. Consenting individuals reported to the outpatient Clinical Research Center of the Brigham and Women's Hospital in Boston.

Blood lead measurement

Blood for lead measurements was taken in special lead-free tubes containing edetate calcium disodium (EDTA) and sent for analysis to ESA Laboratories, Inc., in Chelmsford, Massachusetts. Blood samples underwent room temperature digestion with nitric acid. The resulting solution was centrifuged, and the supernatant was poured into a sample cup and analyzed by Zeeman background-corrected flameless atomic absorption (graphite furnace). The instrument was calibrated before use with National Bureau of Standards blood lead standards materials, and the calibration was rechecked after every 21 samples. Ten percent of samples were run in duplicate; ≥10 percent of the analyses were controls, and 10 percent of the analyses were blanks. A complete calibration check was made after the last specimen was analyzed. All glassware involved in processing specimens was soaked overnight in 20 percent nitric acid and rinsed several times with distilled water to prevent lead contamination. Blood specimens and reagents were aliquoted with micropipettes with disposable polyethylene tips. In tests on reference samples from the Centers for Disease Control and Prevention (CDC), precision (coefficient of variation) ranged from 8 percent for concentrations below 30 μg/dl to 1 percent for higher concentrations. In comparison with a National Bureau of Standards target of 5.7 μg/dl, 24 measurements by this method gave a mean of 5.3 μg/dl with a standard deviation of 1.23.

KXRIF bone lead measurements

Bone lead measurements were taken of each subject's mid-tibial shaft and patella using an ABIOMED KXRIF instrument (ABIOMED, Inc., Danvers, Massachusetts). The physical principles, technical specifications, validation, and quality control procedures of this instrument have been described in detail elsewhere (10, 15, 16), as have those of other KXRIF instruments (9, 17). In short, this instrument uses a 109Cd gamma-ray source to provoke the emission of fluorescent photons from target tissue that are then detected, counted, and arrayed on a spectrum (18). The net lead signal is determined after subtraction of Compton background counts by a linear least-squares algorithm. The lead fluorescence signal is then normalized to the elastic or coherently scattered gamma-ray signal,
which arises predominantly from the calcium and phosphorus present in bone mineral. The unit of measurement so derived is $\mu$g of lead per g of bone mineral ($\mu$g/g). Because the instrument provides a continuous unbiased point estimate that oscillates around the true bone lead value, negative point estimates are sometimes produced when the true bone lead value is close to zero. The instrument also provides an estimate of the uncertainty associated with each measurement that is derived from a goodness of fit calculation of the spectrum curves and is equivalent to a single standard deviation. Although a minimum detectable limit calculation of twice this value has been proposed for interpreting an individual's bone lead estimate (19), retention of all point estimates makes better use of the data in epidemiologic studies (20).

For the present study, 30-minute measurements were taken at the midshaft of the left tibia (representing cortical bone) and at the left patella (representing trabecular bone) after each region had been washed with a 50 percent solution of isopropyl alcohol. The KXRF beam collimator was sited perpendicular to the bone surface for the tibia and at 30° in the lateral direction for the patella.

**Statistical analyses**

Using information collected on smoking since the inception of the study in 1961, cumulative pack-years of smoking were calculated for each individual. The proportion of all visits (dating back to the beginning of the study) in which the individual reported drinking $\geq 2$ alcoholic drinks per day was derived as an index of cumulative alcohol ingestion.

As a check on the quality of the KXRF measurements, tibia and patella bone lead measurements with associated measurement uncertainty estimates of $>10$ and $>15$ $\mu$g/g, respectively, were identified and discarded (21). For all continuous variables, univariate statistics were calculated and statistical outliers were identified by the extreme Studentized deviate (ESD) procedure (22). Subsequent analyses were run with and without the outliers.

Subjects who volunteered for the KXRF measurements were compared with other Normative Aging Study subjects who came for their regularly scheduled visit but who did not volunteer for KXRF measurements, across categories of age, race, current blood lead level, smoking and alcohol ingestion habits, and retirement status.

Among all KXRF participants, levels of blood and bone lead were compared across categories of age, race, current smoking and alcohol ingestion, cumulative smoking and alcohol ingestion, and retirement status, and the significance of differences across categories (excluding “no information” categories) was tested by analysis of variance using values of blood and bone lead that were transformed to achieve normality. For blood lead, the log transformation was used. For tibia and patella lead, prior to log transformation, a value of 35 was added to the measurements (as first described by Kosnett et al. (11)). In addition, because both tibia and patella lead (as well as their log-transformed values) had strong positive correlations with age ($r = 0.38$ ($p < 0.0001$) and $0.31$ ($p < 0.0001$), respectively; see figures 1 and 2), as has been reported in other cross-sectional surveys of community-exposed individuals (10–12, 23), the transformed values of bone lead were analyzed after being adjusted by least squares regression to the mean age.

Multivariate linear regression models were then constructed to predict tibia lead, patella lead, and blood lead. Each began with a saturated model including terms for age, race, education, current smoking status, cumulative pack-years of smoking, current alcohol ingestion, cumulative alcohol ingestion, and retirement status. The need to transform variables to conform to these linear models was assessed by examining generalized additive models (GAMs). A GAM is an extension of the linear regression model that replaces the usual linear function of a covariate by an unrestricted smooth function. The smooth function is estimated and then plotted against the covariate, thus providing an indication of the appropriate transformation to linearity (24).

Final models were obtained by applying a backwards elimination procedure that kept continuous variables with $p$ values $< 0.05$ and whole sets of indicators corresponding to categorical variables if individual indicators in each set had $p$ values $\leq 0.05$. The procedure terminated when all remaining variables met the above criteria or when no additional continuous indicators or set of categorical indicators could be removed without a decrease in the total model $R^2$ of at least 10 percent (whichever came first). Models predicting bone lead incorporated weights based on the inverse of the square of the individual estimates of bone lead uncertainty. In order to test the hypothesis that bone lead contributes significantly to blood lead levels even after control for age and lifestyle factors, both tibia and patella lead were added to the model predicting blood lead before the backwards elimination process.

Measurement uncertainty in an independent variable biases the ordinary least squares estimate of its effect toward the null. Because each estimate of bone lead generated by a KXRF measurement is accompanied by
an individual estimate of measurement uncertainty, we used methods based on Fuller (25) and described elsewhere (16) to derive adjusted parameter estimates and 95 percent confidence intervals that are approximately unbiased in final regression models of blood lead that included bone lead as an independent variable.

RESULTS

Out of the 1,261 Normative Aging Study subjects who were seen for their regularly scheduled visits between 1991 and 1994, a total of 719 participated in the KXRF bone lead study. The mean age was 66.8 years. The most common reason cited for nonparticipation in the study was the inconvenience of going to another facility on a separate day. Participants were similar to nonparticipants with respect to distributions of age, race, education, smoking status, consumption of alcohol, retirement status, and blood lead level (table 1).

Among the KXRF bone lead study participants, blood lead levels had a lognormal distribution with a median and geometric mean of 5 and 5.7 μg/dl, respectively. Four values of tibia lead and one value of patella lead were dropped because of excessive measurement uncertainties. We observed lognormal bone mineral distributions with median and geometric mean values of 20 and 20.8 μg/g, respectively, for tibia lead, and 28 and 29.8 μg/g, respectively, for patella lead.

In bivariate analyses (table 2), blood lead, age-adjusted tibia lead, and age-adjusted patella lead were higher in subjects who were older, black, of the lowest educational level, current smokers, smokers with the most cumulative pack-years, consumers of >2 alcoholic drinks per day, and consumers of the most alcohol over time. Blood lead was also higher among subjects who were retired and subjects who had the highest tibia or patella bone lead levels. Tibia and patella bone lead levels were higher among retirees,
but these differences disappeared after adjusting the bone lead levels for age. The increases in tibia and patella bone lead with age were both relatively linear (figures 1 and 2).

Our initial regression models that contained all the variables of interest are presented in table 3. After backwards elimination, age, level of education, and pack-years of smoking remained as significant predictors of tibia lead (table 4). The same variables, with the addition of retirement, remained as significant predictors of patella lead (table 4). Compared with subjects with graduate or professional school education, sub-

jackets who did not finish high school had mean tibia and patella lead levels that were 11.4 and 12.6 μg/g higher, respectively. Compared with never smokers, smokers with >40 pack-years of smoking had mean tibia and patella lead levels that were 3.6 and 12.2 μg/g higher, respectively.

In the final model of blood lead (table 5), age, being black, and patella lead remained as correlates of higher blood lead, with patella lead accounting for the far greater proportion of the variance in blood lead (a partial R² of 0.15 out of a total model R² of 0.17). Moderate but not the heaviest cumulative smoking...

was associated with lower blood lead levels. A rise in patella lead from the median of the lowest to the median of the highest quintile (13 to 56 \(\mu g/g\)) was associated with a rise in blood lead of 3.6 \(\mu g/dl\) (figure 3). After adjustment for measurement error, this estimate increases to 4.3 \(\mu g/dl\).

DISCUSSION

In this study, we found that community-exposed men had relatively low levels of blood lead (geometric mean = 5.7 \(\mu g/dl\)), paralleling those reported for white males aged 50–69 years in the recent 1988–1991 NHANES III (geometric mean = 4.8 \(\mu g/dl\)) (26). Levels of lead in the tibia were generally higher than had been reported in surveys we conducted earlier in 35 community-exposed adults (10) and 129 construction workers with moderate occupational exposures to lead (23), as well as an investigation by Kosnett et al. (11) of 101 community-exposed subjects studied with a similar KXRF instrument. This discrepancy probably reflects, at least in part, the older age distribution of the current study population, because bone lead levels have been found in all studies to be directly correlated with age. Nevertheless, the interindividual variation was fairly large throughout the age spectrum in this study and reflected the wide variation in environmental lead exposure. In comparison, Gerhardsson et al. (27, 28) studied 30 retired lead smelter workers with similar age distribution (mean = 67.9 years), and they found a median KXRF-measured tibia lead level of 39.3 \(\mu g/g\), substantially higher that the median tibia lead level of 20 \(\mu g/g\) observed in the Normative Aging Study.

We are not aware of previous studies that have reported on patella lead levels among community-exposed adults. We examined patella lead levels because of evidence that cortical bone (tibia) and trabecular bone (patella) are likely to have different kinetics and, therefore, possibly different toxicologic implications (6). In our study of construction workers (23), patella lead levels were lower than in the current study; however, in both studies, patella lead levels were roughly 50 percent higher than tibia lead levels.

The finding of a significant association of pack-years of smoking with bone lead parallels the findings from an autopsy study (29) and a smaller KXRF study by Kosnett et al. (11) and reflects the ability of bone lead, rather than blood lead, to signify cumulative lead
exposure. Smoking entails lead exposure by a direct contribution from tobacco (which is contaminated by atmospheric lead deposition and lead arsenate pesticides) (30, 31), increased hand-to-mouth activity, and possibly the enhanced permeability of a smoke-exposed respiratory tract (32).

Retirement was found to be associated with higher patella bone lead levels in this study. This is unlikely to represent any particular "exposure" associated with retirement; instead, retirement is probably a proxy for age. This would explain why the parameter estimate for age is smaller in the model of patella lead than in the model of tibia lead, which is the opposite of what we have found in other studies in which retirement status was not considered (26, 33).

Fewer years of education had a very strong association with higher bone lead levels in our study. Although a number of studies have controlled for years of education (or social class), in examining the relations of lead to mental development, few investigators have specifically studied the impact of education on lead biologic markers in relation to other environmental variables. Social class was found to be negatively correlated with blood lead levels even after adjustment for age, sex, smoking and drinking habits, age of dwelling, and geographic location in the United Kingdom (34). Moore et al. (35) found that an association between social class and blood lead levels in Scotland was probably related to a close relation between social class and lead levels in tap water. Muldoon et al. (36) did not find an association between educational level and blood lead levels among elderly women in the United States, while Kosnett et al. (11) did not find a relation between educational level and bone lead levels in their smaller KXRF study. In our study, education may be serving as a proxy for unmeasured cumulative environmental exposures, such as home exposure to lead in dust and drinking water, home proximity to vehicular traffic, and/or occupational lead exposures.

The dominant contribution of trabecular bone (patella) to blood lead is perhaps the finding of this study with the greatest public health significance. Studies that have used stable isotope tracers (37, 38) and KXRF measurements of occupationally exposed workers after their retirement (39) have demonstrated a slow decline in blood lead levels, which suggests that circulating lead is being supplied by a long-term storage compartment, most likely bone. Ours is the first epidemiologic study of which we are aware that
employed direct KXRF bone lead measurements to provide evidence implicating bone lead as a significant source of circulating lead in adults from the general population. The fact that in our final regression model blood lead was predicted by lead in the patella (trabecular) rather than the tibia (cortical), despite the greater measurement error associated with patella lead measurements, may reflect the higher turnover rates, permeability, and vascularity of trabecular bone.

Because aging-associated bone demineralization can be expected to increase the liberation of bone lead stores into circulation, as has been suggested by a study that found increased blood lead levels among postmenopausal as opposed to premenopausal women.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter estimate</th>
<th>95% CI</th>
<th>P value</th>
<th>Partial R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.05</td>
<td>-0.09, -0.01</td>
<td>0.0176</td>
<td>0.0053</td>
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<tr>
<td>Black†</td>
<td>2.27</td>
<td>0.34, 4.20</td>
<td>0.0217</td>
<td>0.0062</td>
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<tr>
<td>Cumulative smoking (pack-years)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–20</td>
<td>-0.86</td>
<td>-1.58, -0.14</td>
<td>0.0198</td>
<td>0.0048</td>
</tr>
<tr>
<td>21–40</td>
<td>-0.93</td>
<td>-1.73, -0.13</td>
<td>0.0225</td>
<td>0.0078</td>
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<tr>
<td>&gt;40</td>
<td>0.04</td>
<td>-0.71, 0.80</td>
<td>0.8086</td>
<td>0.0000</td>
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<td>Patella lead (μg/g)</td>
<td>0.083</td>
<td>0.068, 0.098</td>
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<tr>
<td>Total model R²</td>
<td>0.17</td>
<td></td>
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</tbody>
</table>

* CI, confidence interval.
† Compared with whites.
‡ Compared with never smokers.


(40), this finding raises the question as to whether long-lived stores of lead in bone are a risk factor for significant toxicity in the elderly. Indeed, even low blood lead levels have been reported to be associated with decreased cognitive performance among elderly women (36); one wonders whether the association would have been stronger with bone lead. Moreover, a recent study (41) that demonstrated that levels of lead in bone, rather than whole blood, best correlate with levels of lead in plasma (the most biologically active but difficult to measure fraction of lead in blood) further suggests that the contribution of lead burden to toxicity in the elderly cannot be adequately assessed by relying on whole blood lead measurements as dose markers.

On the other hand, the cross-sectional design of the current investigation limits our ability to causally relate bone lead stores to circulating lead levels. Further research is necessary to determine whether release of lead stored in bone contributes materially to circulating lead and morbidity, and whether bone stores of lead represent a threat to public health. In the mean-
time, efforts should continue to decrease overall lead exposure in the general population.

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