

# INFLUENCE OF DIETARY ASCORBIC ACID INTAKE ON LEAD MOBILIZED FROM BONE AMONG MIDDLE-AGED AND ELDERLY MEN: THE NORMATIVE AGING STUDY

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## Abstract

The skeleton is the major endogenous source of lead to circulating blood, particularly during periods of accelerated bone turnover and mineral loss, such as occurs during pregnancy and post-menopausal osteoporosis. Animal studies have demonstrated that ascorbic acid (AA) decreases the intestinal absorption of lead and increases the renal clearance of lead. To assess the influence of dietary AA intake on lead that has been released from bone, we analyzed the cross-sectional relation of bone lead to urinary lead (UPb) in 299 men (mean age 66 years; range 52 to 84 years) using multiple regression analysis with stratification by terciles of AA intake. The independent contribution of bone lead to UPb increased with increasing AA. Conversely, the positive relation of blood lead to UPb decreased with increasing AA. Since lead excreted in urine is derived from plasma, our findings suggest that bone, independent of blood, is a source of lead in plasma among middle-aged and elderly men in the general population and the release of lead from bone may be modified by dietary intake of AA. AA may have a role as a public health recommendation for the reduction of long-term risks associated with low-level chronic lead exposure in aging populations.

## INTRODUCTION

AA has been suggested to act as a chelating agent, which enhances the urinary elimination of lead from the body (Dhawan et al. 1988; Flora and Tandon 1986). Low dietary AA intake was found to significantly increase blood lead level (BPb) among middle-aged and elderly men (Cheng et al. 1998), whereas, NHANES III data showed only serum AA, but not dietary AA intake, was inversely related to BPb among adults and youths (Simon and Hudes 1999). The skeleton is the major endogenous source of lead to circulating blood, particularly during periods of accelerated bone turnover and mineral loss, such as occurs during pregnancy (Gulson et al. 1998) and post-menopausal osteoporosis (Silbergeld et al. 1988). Given that an increase in bone resorption is a characteristic of aging in both men and women, resorptive losses may also contribute to longitudinal declines in bone lead, particularly in older populations. Aging-associated release of bone lead into the circulation is, in fact, a potentially important source of soft-tissue lead exposure and toxicity. Data from a longitudinal analyses has shown that bone lead levels (particularly those in the patella, a trabecular bone) decreased over a 3-year follow-up interval (Kim et al. 1997). UPb originates from plasma lead that has been filtered at the glomerular level; thus, UPb adjusted for glomerular filtration rate serves as a marker of plasma

lead. This study was to assess the influence of dietary AA intake on the release of lead from bone among middle-aged and elderly men environmentally exposed to lead.

## **METHODS**

### **Subjects and Measurements**

This investigation took advantage of an ongoing longitudinal cohort study of aging, the Normative Aging Study (NAS), established in 1961. The study cohort initially consisted of 2,280 men from the Greater Boston, Massachusetts, area. The men were 21-80 years upon enrollment. All participants were free of known chronic medical conditions at the time of enrollment; participants were excluded if they had any history of cancer, recurrent asthma, sinusitis, bronchitis, diabetes, gout, or peptic ulcer. Those with a systolic blood pressure of >140 mmHg or a diastolic blood pressure of >90 mmHg were also disqualified. Since their enrollment in 1961-1968, participants have been reevaluated at 3- to 5-year intervals by a detailed core examination including collection of medical history information, routine physical examinations, laboratory tests, and questionnaires. Beginning in 1987, a 24-hr urine specimen was obtained at each subject's regularly scheduled examination. In addition, a blood sample for lead analysis has been collected at each visit since 1988. Lead in urine and blood was measured by inductively coupled plasma mass spectroscopy and graphite furnace atomic absorption spectroscopy and, respectively. Beginning in 1991, NAS participants were recruited for a substudy of K-x-ray fluorescence (KXRF) bone lead measurement in cortical (tibia) and trabecular (patella) bone. Creatinine in serum and in archived (frozen) 24-hr urine samples was measured by the Jaffe rate method by the Beckman Creatinine Analyzer 2. The methods used here are described in detail elsewhere (Tsaih et al. 1999). Dietary intake of nutrients was assessed with a self-administered semiquantitative food-frequency questionnaire adapted from a well-validated questionnaire used elsewhere (Salvini et al. 1989). We identified 380 NAS participants who were participants in the KXRF bone lead substudy and had 2 consecutive valid 24-hr urine specimens. A valid 24-hr urine specimen was defined as having a volume of 750 - 2900 ml and a collection time of 20 - 28 hr to ensure the completeness of our samples. All research performed in our study was approved by the Human Research Committees of Brigham and Women's Hospital (Boston, MA) and the Department of Veterans Affairs Outpatient Clinic (Boston, MA).

### **Statistical methods**

Outliers identified by the generalized ESD many-outlier procedures were excluded from all analyses. Levels of urinary lead were  $\log_e$  transformed to normalize the distribution of values. To assess the influence of AA on the release of lead from bone, we analyzed the relation of bone lead and BPb to UPb using multiple regression analysis with stratification by terciles (low/medium/high) of AA. To minimize the influence by variability in renal function, we excluded all subjects with renal impairment (serum creatinine levels of >1.5 mg/dl), and adjusted all models for creatinine clearance (CCr) as an estimate of glomerular filtration function. CCr (ml/min) was calculated as total amount of urine creatinine over 24 hr (mg)/[serum creatinine concentration (mg/ml)  $\times$  collection time (min)]. Age, body weight, cumulative cigarette smoking (pack-years), alcohol consumption (g/day), and education level were included in all regression models as potential confounders. To minimize extraneous error in estimating nutrient intake due to individual differences in total food intake and to reduce potential confounding by total food intake, nutrients were adjusted for total energy intake. Chi-square test of homogeneity was used to test whether the regression estimates vary according to AA terciles. Patella lead (TBoPb) and

tibia lead (CBoPb) - theoretically representing different bone lead kinetics, yet highly correlated with each other - were assessed separately in multiple linear regression models. All analyses were performed in SAS 6.12.

## RESULTS AND DISCUSSION

**Table 1.** Selected characteristics of 299 middle-aged and elderly male participants in the present study

Variable	Mean $\pm$ SD
Age (years)	66.5 $\pm$ 6.0
Urine lead ( $\mu\text{g}/\text{day}$ )	4.7 <sup>†</sup> $\pm$ 1.6
Blood lead ( $\mu\text{g}/\text{dL}$ )	5.7 $\pm$ 3.35
Patella lead ( $\mu\text{g}/\text{g}$ )	29.6 $\pm$ 14.9
Tibia lead ( $\mu\text{g}/\text{g}$ )	20.4 $\pm$ 9.6
Creatinine clearance rate (ml/min)	85.8 $\pm$ 23.0
Alcohol consumption (gm/day)	13.3 $\pm$ 17.8
Cigarette smoking (pack-years)	18.7 $\pm$ 23.2
Ascorbic acid intake (mg/day) <sup>‡</sup>	291.6 <sup>†</sup> $\pm$ 308.7
Calcium intake (mg/day) <sup>‡</sup>	833.7 <sup>†</sup> $\pm$ 365.5

SD, standard deviation  
<sup>†</sup> Geometric mean  $\pm$  geometric standard deviation  
<sup>‡</sup> Total energy-adjusted dietary intake

Eighty-one subjects out of the 380 eligible subjects were excluded because they were either identified as outliers, with a reduced renal function (serum creatinine concentrations of  $> 1.5$  mg/dl), or with incomplete data. The selected characteristics of the remaining 299 subjects are summarized in Table 1. As expected, lead measures (blood, bone and urine) were moderately correlated ( $r = 0.2$  to  $0.6$ , all  $p < 0.05$ ). AA intake was significantly associated with Age, BPb and calcium intake ( $r = 0.20$ ,  $-0.16$  and  $0.19$ , respectively). As reported previously, although BPb was a consistently important

**Table 2.** Multivariate-adjusted parameter estimates of 24-hr urine lead levels and blood lead levels in association with blood lead and bone lead by ascorbic acid tertiles

	Terciles of ascorbic acid		
	1 <i>b</i> (SE)	2 <i>b</i> (SE)	3 <i>b</i> (SE)
<i>Linear regression model of 24-hr urinary lead levels</i> <sup>*</sup>			
Model 1			
Blood lead	0.0815 <sup>†</sup> (0.0108)	0.0641 <sup>†</sup> (0.0132)	0.0703 <sup>†</sup> (0.0134)
Model 2			
Blood lead	0.0723 <sup>†</sup> (0.0117)	0.0518 <sup>†</sup> (0.0136)	0.0532 <sup>†</sup> (0.0143)
Patella lead	0.0054 (0.0029)	0.0083 <sup>†</sup> (0.0031)	0.0091 <sup>†</sup> (0.0032)
Model 3			
Blood lead	0.0787 <sup>†</sup> (0.0114)	0.0549 <sup>†</sup> (0.0134)	0.0633 <sup>†</sup> (0.0143)
Tibia lead	0.0033 (0.0044)	0.0125 <sup>†</sup> (0.0050)	0.0066 <sup>†</sup> (0.0047)
<i>Linear regression model of blood lead</i> <sup>‡</sup>			
Model 4			
Patella lead	0.1053 <sup>†</sup> (0.0231)	0.0737 <sup>†</sup> (0.0213)	0.0953 <sup>†</sup> (0.0210)
Model 5			
Tibia lead	0.1258 <sup>†</sup> (0.0370)	0.1018 <sup>†</sup> (0.0360)	0.1193 <sup>†</sup> (0.0316)

SE, standard error  
<sup>\*</sup> 24-hr urinary lead was log<sub>e</sub> transformed and each model was adjusted for age and CCr  
<sup>†</sup> Each model was adjusted for age  
<sup>‡</sup>  $p < 0.05$

determinant of UPb, bone lead was shown to contribute independently to UPb in the study population (Tsaih et al. 1999). Men in the lowest AA tercile had the strongest BPb-UPb association (Table 2). On the contrary, the stronger independent TBoPb-UPb and CBoPb-UPb associations were observed in the upper two AA terciles and no independent CBoPb-UPb association was observed in the lowest AA tercile. Furthermore, the strongest bone lead-BPb associations were observed in the lowest AA tercile. Although the chi-square test failed to show any significant difference of these associations across

AA terciles, this test is known to lack power to detect the heterogeneity.

Our findings of stronger relationships of BPb to UPb and of bone lead to BPb in men with low AA intake and a stronger independent relationship of bone lead-UPb in men with greater AA intake are consistent with previous findings that AA reduces lead absorption, increases the elimination of lead from soft tissues (Flora and Tandon 1986), and enhances the efficacy of chelation therapy (Dhawan et al. 1988). Lead in diet, in addition to bone lead and BPb, may contribute substantially to lead in urine for individuals without occupational exposure. Our study was limited by the fact that dietary intake of lead was not measured and we were unable to distinguish between lead from the skeleton and lead from dietary sources. Nevertheless, in an aging non-occupationally population like the NAS, with relatively high bone lead levels, the influence of diet should be minimal. It is possible that higher AA intake may represent better social economic status or healthier lifestyles. However, none of the other variables (body weight, education level, alcohol consumption, cumulative cigarette smoking, calcium intake) predicted UPb, nor did their inclusion in the models change the regression coefficients of TBoPb, CBoPb, or BPb to a notable extent. In conclusion, since lead excreted in urine is presumed to be derived from plasma, our findings suggest that bone, independent of blood, is a source of lead in plasma among middle-aged and elderly men in the general population and the release of lead from bone may be modified by AA intake. AA may have a role as a public health recommendation for the reduction of long-term risks associated with low-level chronic lead exposure in aging populations. Further investigation is needed.

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