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Bone, blood and semen lead in men with environmental and moderate occupational exposure

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Abstract
Lead (Pb) in blood, bone, and semen was measured in 162 to 186 environmentally exposed men from Mexico City, aged 19 – 48. Semen Pb was measured by inductively coupled plasma mass spectrometry, blood Pb by atomic absorption spectrometry and bone Pb by K X-ray fluorescence. Mean Pb levels in blood, semen, tibia (cortical) and patella (trabecular) bone were 12 μg/dl, 2.7 μg/l, 13 μg/g, and 20 μg/g, respectively. Semen Pb was determined by blood Pb and patella Pb. Determinants of higher tibia Pb were age, living near industry in which Pb is used, and a high occupational Pb exposure index. Higher patella Pb was predicted by age, higher traffic density near home, a high index of occupational exposure to Pb and a greater number of cigarettes smoked per day in the year prior to the study. Blood and bone Pb results are consistent with findings in other populations. Semen results provide new information on the semen-bone Pb relationship. Bone, especially trabecular one, proved to be a significant endogenous lead source for blood and semen burdens in reproductive aged men.

Keywords: *Bone lead, blood lead, semen lead, environmental lead, K X-ray fluorescence*

Introduction

In many parts of the world, Pb exposure is still common, affecting the health of men, their partners and their offspring even at relatively low levels. Epidemiological studies have shown conflicting results regarding Pb exposure, sperm abnormalities and male fertility, especially at lower doses (Robins et al. 1997). While some studies have found no association (Coste et al. 1991; Xu et al. 1993) or small and reversible adverse effects (Viskum et al. 1999), it is also believed that long-term exposure to Pb may produce morphological and functional sperm changes (Assennato et al. 1987; Lerda 1992; Alexander et al. 1996) and may reduce male fertility (Lin et al. 1996). Other studies have found significantly higher blood Pb levels in infertile men compared with fertile men (Jöckenhovel et al. 1990). An indirect effect of male

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Pb exposure may be an increased risk of spontaneous abortion, although this has been suggested at higher exposures, such as those found in occupational contexts (Lindbohm et al. 1991a,b, 1992; Savitz et al. 1994). Experimental studies in rats and monkeys have suggested that chronic Pb exposure may harm spermatozoa and may have subtle effects on pituitary and Sertolly cell function (Foster et al. 1993; Marchlewicz et al. 1993).

Most of the information on Pb sources and levels comes from studies measuring blood Pb, a short-term exposure measurement. In this study we explored the contribution of endogenous sources of lead (blood and bone) to lead levels observed in semen among men who had been subject to long term, low to moderate non-occupational lead exposure.

Materials and methods

Study population

In this cross-sectional study, participants were recruited between 1996 and 1998 at the National Institute of Perinatology in Mexico City at the outpatient clinic and blood bank. Participants were of low or medium socioeconomic status, and most of them were not engaged in occupations involving primary Pb exposure.

Those who did not agree to participate answered 5 questions about Pb exposure and the reason for declining. The ones who agreed to participate signed an informed consent form, filled out a questionnaire about expected risk factors for Pb exposure, and had their Pb levels measured in semen, blood, tibia and patella. Study subjects received their results and the appropriate counseling regarding lead exposure.

Questionnaires

A structured questionnaire was used to collect information about the following characteristics: (1) sociodemographics (2) lifestyles (3) reproductive history and (4) sources of exposure to Pb. In terms of lifestyles, we explored tobacco and alcohol consumption. With regard to tobacco use, we asked about present and past smoking habit and duration and number of cigarettes per day in each case. As for alcohol consumption, we evaluated consumption frequency (abstainers, less then once a month but at least once a year, from two to three times a month and one or more times a week) and the number of drinks consumed each time. The following sources of environmental and indoor exposure to Pb were identified: type of vehicular traffic existing in the place of residence (intense, intermediate and low), time residing in Mexico City (in years), location of residency in relation to industries that use Pb, current and past use of traditional lead-glazed ceramics coated with Pb glaze, and the overall time during which food consumed in the last month was either prepared or stored in lead-glazed ceramic ware. We also obtained histories of occupations with potential exposure to Pb. Participants were asked if they had ever been occupationally exposed to Pb, in what type of occupation and for how long.

Blood Pb measurement

Blood samples were drawn and stored in heparinized Pb-free tubes at 4°C and were analyzed with a graphite furnace atomic absorption spectrophotometry instrument (Perkin Elmer 3000) at the metals laboratory of the American British Cowdray (ABC) Hospital in Mexico City. The laboratory standardization program of the Wisconsin State Laboratory of Hygiene, Madison, Wisconsin, provided external quality control specimens varying from 2 to 88 μg/dL.

Our laboratory maintained acceptable precision and accuracy during the study time (correlation = 0.98; mean difference 0.71 μg/dL; standard deviation (SD) = 0.68).

Semen Pb measurement

Study subjects gave a semen sample by masturbation. They were instructed on how to wash their hands first and then their genitalia with soap and water and finally to rinse with deionized water to avoid Pb contamination. Semen samples were stored at 4°C until they were analyzed at the Metals Laboratory of the Harvard School of Public Health, Boston, MA.

All samples were handled in a Class 100 clean hood. Semen samples (1 ml) were prepared by digestion with nitric acid (1 ml) (Optima, Seastar Chemical Co., Pittsburgh, PA) at room-temperature and subsequent dilution with distilled deionized water (5 ml) (McLaren et al. 1987).

Samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Sciex Elan 5000, Perkin Elmer, Norwalk, CT), with standard instrument operating and data collection parameters, using the isotope dilution (ID) procedure. The mass spectrometer setting and nebulizer flow rate were optimized to give a maximum peak intensity for Pb. A solution of enriched isotope spike ^{206}Pb, National Institute of Standard and Technology Standard Reference Material (NIST SRM) 983 were then mixed with the sample for ID-ICP-MS analysis (1 – 3). Data given were the average of 5 replicate measurements by the instrument (Viczian and Barnes 1990; Amarasiriwardena et al. 1994).

Quality control (QC) and quality assurance procedures included analyses of procedural blanks to monitor contamination and NIST SRM 955a (Pb in blood, QC standard for semen samples), spiked samples, and NIST SRM 1643d (trace elements in water) to monitor the accuracy and recovery rates of the procedure for each analytic batch. Recovery of these QC standards is between 90% and 110% in our laboratory.

The calculated detection limit for Pb analysis by this procedure is 0.2 ng per ml of semen, which was estimated by multiplying the standard deviation of the 10 instrumental measurements of the method blank by 3 and multiplying that value by the sample dilution factor.

Bone Pb measurement

Bone lead levels were measured at the patella and tibia *in vivo* with a KXRF instrument. Thirty-minute measurements were taken at the midshaft of the tibia and at the left patella after each region had been washed with a 50% solution of isopropyl alcohol. For that purpose, a ^{109}Cd gamma ray KXRF instrument, constructed at Harvard University and installed in a BRIMEX II Research Center at the ABC Hospital in Mexico City, was used. Aro et al. (1994) have already described the physical principles, technical specifications and steps needed for the validation of this instrument which uses a fixed gamma ray source to cause the emission of fluorescent photons from target tissue and which, upon being detected, are transformed into a spectrum. The results are reported as micrograms of lead per gram of bone mineral (μg Pb/g).

Data analysis

A univariate analysis was conducted on all variables to describe their distribution and check for outliers. Two semen Pb values were found to be outliers, i.e., 2 standard deviations above the upper limit (25.88 μg/l). Since this was most probably due to contamination, these values were excluded from further analyses. Blood and semen Pb levels were log-e transformed for statistical analysis. TPb or PPb measurements were discarded if their associated measurement uncertainty was greater than 10 or 15 μg/g, respectively. This resulted in the elimination of 1 patella measurement and 14 tibia Pb measurements.

We conducted a bivariate analysis for semen, blood, tibia and patella Pb and each potential predictor. Finally, a multivariate model was created for each dependent variable by including all of the independent variables that reached a statistical significance of $P < 0.15$ in the bivariate analyses. A backward elimination procedure was then applied to discard variables that did not reach statistical significance at the $P < 0.05$ level or did not significantly affect the rest of the coefficients in the model (did not produce a larger than 10% change). Because both bone and blood lead were independent predictors of semen lead and were also in the same causal pathway, we used Principal Component Analyses (PCA) to transform these variables into a set of uncorrelated components. The primary objective of using this procedure was to statistically assess the independent contribution of each component to semen lead concentrations. All statistical analyses were conducted using STATA (Stata Statistical Software, Release 7.0, Stata Corporation College Station, TX). Results on the analyses of the relationships between seminal characteristics and all Pb biomarkers studied will be reported in a future publication.

Results

Of the 417 men invited to take part in the study, 155 did not participate. The main reasons for not participating were lack of time (38%), they could not leave their jobs to be studied (28%) or they did not meet all of the eligibility criteria (16%). Men who did not participate in the study did not differ significantly from those who participated in terms of age (mean age of non-participants = 30) or recalled exposure to the main Pb sources studied.

The age of the 262 participants ranged from 19 to 48 years, with a mean of 33 years. On average participants had lived in Mexico City for 31 years, with a range from 4 years to 48 years. Participants on average had lived 94% of their life in Mexico City. Low or no Pb exposure occupations were the most prevalent reported occupations in our study population (Table I). Blood-lead levels were distributed between a minimum value of 2.1 and a maximum of 32.1 μg/dl, with a mean of 11.98 μg/dl. Semen Pb levels varied between 0.02

Table I. Occupation rating, distribution and mean duration per category in terms of Pb exposure in Mexican men.

	Occupational Pb exposure		
	Negligible	Low	High
	Office worker (81) Other[a] (58)	Street vendor (34) Painter (33) Fine art painter (12) Driver (60) Electrician (4) Plumber (14) Welder (28) Oil refinery worker (2) Road paver (2) Sewage worker (6)	Pb smelter worker (8) Battery refurbishing shop worker (2) Ceramics industry worker (3) Gas station attendant (9) Mechanic (29) Print shop worker (19)
Total (N)	(139)	(195)	(70)
Mean months worked	34	87	29

[a]This category included several jobs of no known Pb exposure. (Number of men who ever worked at each job).

and 19.42 μg/l, with a mean of 2.66 μg/l. Lead values in trabecular and cortical bone were distributed with means of 20.32 μg Pb/g of bone mineral (SD = 14.9) and 14.5 (SD = 10.09) respectively (Table II).

Results of the bivariate analyses between all Pb biomarkers and independent variables studied are shown on Table III. For the most part, positive bivariate associations were seen between the different variables studied, but only a few of them reached the statistical cutoff point to be included in the multivariate analyses. Variables for occupation and proximity of housing to industries that use Pb were grouped in order to increase power. Proximity of housing to a Pb using industry was significantly associated with higher tibia Pb levels. After categorizing occupation into non (0), low (1) or high (2) Pb exposure, an index was created by multiplying this number by the number of months exposed. This index of occupational exposure was a predictor of both tibia and patella Pb levels.

Of all the smoking variables studied, only the association between smoking at work and blood Pb levels and the association between cigarettes smoked per day during the last year and patella Pb levels remained significant after controlling for other variables. Age and time lived in Mexico City were strongly correlated, and both variables significantly predicted higher bone Pb levels. Because the associations with age were stronger and more significant, the final bone Pb models included age and were not adjusted by time lived in Mexico City. Final linear multivariate models of blood, tibia, patella and semen Pb predictors are shown in Table IV. Blood Pb was increased by the days per week of cooking food in lead-glazed ceramics, patella Pb levels and smoking at work. Tibia Pb levels also predicted blood Pb levels (coefficient: 0.0079, $P(t) = 0.010$), but not as strongly and significantly as patella Pb levels did.

Tibia Pb levels were associated with age, and with variables indicating a Pb-using industry near the house of the participant and if the participant recalled Pb exposure in his current or past occupations. Patella Pb levels were also associated with age and recalled occupational exposure and by the mean number of cigarettes smoked per day during the 12 months preceding the study. Although history of occupational exposure was marginally associated ($P = 0.077$), it was kept in the final model because of its influence on the other coefficients.

Semen Pb levels were statistically predicted only by blood Pb levels and by patella Pb levels, this last variable was included in the model as a dummy variable indicating values above the median. Using PCA, 3 components were generated: the first explained 68% of the variance and it measured bone lead; the second, which accounted for 22% of the variance, reflected high tibia Pb and low patella Pb levels; the third represented 10% of the variance and was a measurement of blood Pb. When the PCA generated variables were included, a significant association was observed between component 1 and component 3, suggesting that blood and bone lead may have an independent contribution on seminal lead.

Table II. Blood, bone and semen Pb levels and their correlations in Mexican men.

		Levels				Correlations			
Biomarker	N	Min.	Mean	Median	Max.	BPb	SPb	TPb	PPb
Blood (μg/dl)	186	4.5	11.98	10.8	40.2	1.0			
Tibia (μg Pb/g bone)	185	ND	14.51	13.26	44.71	0.20*	0.11	1.0	
Patella (μg Pb/g bone)	183	ND	20.32	19.30	66.33	0.33**	0.13	0.48**	1.0
Semen (μg/l)	162	0.02	2.66	1.24	19.42	0.20*	1.0		

BPb: blood lead, SPb: semen lead, TPb: tibia lead, PPb: patella lead. $*P < 0.05$, $**P < 0.001$.

Table III. Statistical significance of the bivariate relationships between blood, bone and semen Pb and their predictors in Mexican men.

Independent variables[a]	Blood Pb	Tibia Pb	Patella Pb	Semen Pb
Age (continuous)	-	↑↑	↑↑	-
Past use of Pb glazed ceramics	↑↑	-	↑	↑↑
Current use of Pb glazed ceramics	↑↑	-	-	↑↑
Ate canned food	-	↑	-	-
Chewed on pencils	↑↑	-	-	↑↑
Smokes	↑	-	-	-
Used to smoke	-	-	-	-
Smokes at work	↑↑	-	-	-
Current cigarettes smoked/day (continuous)	-	-	↑	-
Cigarettes smoked/last year (continuous)	-	-	↑↑	-
Drinks alcohol	-	-	-	-
Glasses of alcohol/week (continuous)	↑	-	-	-
Age when started working (continuous)	↑↑	-	-	↑↑
Time working in current job (continuous)	↑↑	-	-	-
Worked in an office[b]	-	↑	-	-
Worked as painter[b]	-	-	-	-
Worked as artistic painter[b]	-	-	-	-
Worked as driver[b]	-	-	-	-
Worked as electrician[b]	-	-	-	-
Worked as welder[b]	-	-	-	-
Worked in oil refinery[b]	-	-	-	-
Worked with sewage[b]	-	-	-	-
Worked as street vendor[b]	-	-	-	-
Worked paving roads[b]	-	-	-	-
Worked as plumber[b]	-	-	↑	-
Worked at battery refurbishing shop[b]	-	-	-	-
Worked at Pb smelter[b]	-	-	-	-
Worked in ceramics industry[b]	-	-	-	-
Worked at gas station[b]	↑	↑	-	-
Worked as a mechanic[b]	-	↑	↑↑	-
Worked in printing shop[b]	-	-	-	-
Time occupationally exposed to Pb (continuous)	↑↑	↑↑	↑↑	↑↑
Gas station near house	-	↑	-	-
Printing shop near house	-	↑↑	-	↓
Paint factory near house	-	-	-	↓↓
Battery refurbishing shop near house	-	-	↑↑	-
Heavy traffic near house	-	-	↑↑	-
House was painted in last 3 months	-	-	-	↑↑
Recalled Pb exposure in any way	↑↑	↑↑	↑↑	-

[a]All variables are dichotomous (unless otherwise indicated). [b]Currently works or ever worked. Positive associations: ↑ $P \leqslant 0.05$, ↑↑ $0.05 < P \leqslant 0.15$. Negative associations: ↓ $P \leqslant 0.05$, ↓↓ $0.05 < P \leqslant 0.15$. No association: - $P > 0.15$.

Discussion

Even though the mean blood Pb level of this population was above 10 μg/dl, levels found for each biomarker were lower than expected considering the participants' place of residence, age and occupations (drivers, painters, mechanics, gas station attendants, etc.). Levels were also expected to be higher, considering the levels found in similar populations (Assennato et al. 1987; Alexander et al. 1998; Jöckenhovel et al. 1990; Chia et al. 1994; Robins et al. 1997; Telisman et al. 2000) (Table V). The lower than expected blood Pb levels found in this

Table IV. Multivariate linear regression models for Pb predictors of blood, semen and bone in Mexican men.

Dependent Variables	Independent Variables	Coefficient	CI 95%	R^2	N
LBPb (μg/dl)	Prepares food in lead-glazed ceramics[a]	0.091	0.05, 0.13	0.21	174
	Patella Pb[a]	0.01	0.005, 0.014		
	Smokes at work (yes, no)	0.14	− 0.001, 0.279		
LSPb (μg/l)	Blood Pb[a]	0.044	0.014, 0.068	0.101	157
	Larger than median patella Pb vs. Lower than median patella Pb	0.405	0.068, 0.743		
TPb (μg/g bone)	Age[a]	0.29	0.043, 0.544	0.09	181
	Nearby Pb using industry (yes, no)	3.49	0.202, 6.64		
	Occupational exposure[a,b]	0.014	0.006, 0.021		
PPb (μg/g bone)	Age[a]	0.87	0.547, 1.193	0.21	179
	Occupational exposure[a,b]	0.009	− 0.001, 0.019		
	Cigarettes/day during last year[a]	0.54	0.212, 0.866		

CI 95% : 95% confidence interval. [a]Continuous variables. [b]This variable was generated considering type of exposure (high = 2, intermediate = 1, none = 0) multiplied by continuous time of exposure in each category.

study's population might be due to the fact that many of the men considered somehow occupationally exposed, such as drivers, were no longer exposed to leaded gasoline, which was formerly the main source of Pb exposure in Mexico and has gradually declined since 1988 (Cortez-Lugo et al. 2003).

Semen Pb levels reported in the literature in other non-occupationally exposed men were from two to five times greater than the mean level found here (Table V), except for one group of 18 non-occupationally exposed men whose mean semen Pb level was very similar to the mean semen Pb level of the men in this study (Assennato et al. 1987).

In the past, determination of Pb in plasma, serum, and semen samples have been confounded by analytical and methodological difficulties, including poor analytical sensitivity and exogenous contamination since Pb levels in these samples are very low. Using strict contamination control protocols and highly sensitive instrumentation such as ICP-MS has enabled us to make reliably accurate measurements of Pb in these samples. Due to our highly controlled sample collection protocol, sample analysis protocols and use of ICP-MS for the analysis enables us to measure Pb in semen samples low as 0.2 ng/ml. The correlation found between transformed blood Pb and semen Pb was statistically significant and higher in this study than in the study done by Alexander et al. (1998) (p. 465), but it was lower than that found by Telišman et al. (2000) (p. 51).

Other studies have found significant associations between blood, semen and bone Pb and smoking and drinking habits. In a study done from 1982 to 1983, blood Pb was measured in 6437 men and women. A relationship was found between alcohol consumption and blood Pb levels, but no dose-response relationship could be found between the number of cigarettes smoked and blood Pb. Grasmick et al. (1985) Our study revealed that alcohol consumption may increase blood Pb levels and that smokers and, to a lesser degree, former smokers have higher blood Pb levels than non-smokers.

Age was an important bone Pb predictor, as has been seen in other studies in men (Watanabe et al. 1994). Results very similar to ours were seen in a study of carpenters in regard to patella lead and age. Age was not as important in the tibia model in our study as it was in the carpenters study, but this might be explained by the age difference, since the mean age for the carpenters was 48.5. Another explanation could be that the accumulation of bone lead is determined not only by time, but by intensity of exposure, as measured in this study by

Table V. Summary of results of studies on blood and semen Pb levels and correlations in occupationally and non-occupationally exposed men.

Author (year)	Country	Occupational exposure (N)	PbB range (mean) μg/dL	PbS range (mean)μg/L	Correlation PbB-LPbS (p)	Age range (mean)
Farias (2003)	Mexico	Non to moderate (160)	4.5 – 40.2 (10.8)	0.08 – 19.42 (2.66)	0.27 (0.0005)	19 – 48 (33)
Telišman (2000)	Croatia	No (35)	6.7 – 20.8 (10.9)	4.2 – 16.6 (8.6)[a]	0.57 (0.0001)	20 – 43 (31)
		Slight to moderate (83)	11.9 – 65.9 (38.7)	6.5 – 48.3 (15.3)[a]		20 – 43 (30)
Alexander (1998)	Canada	Yes (81)	5 – 58 (22.8)	1 – 176 (19)	0.19 (0.10)	(39)
Robins (1997)	South Africa	Yes (97)	28 – 93 (53.8)	10 – 870 (96)	NI	(38.3)
Chia (1994)	Singapore	No (184)	(7.09)	(12.98)	NI	24 – 54 (34.8)
Jockenhövel (1990)	Germany	No Infertile (172)	(11.18)	NI	NI	NI
		No Fertile (18)	(5.6)	NI	NI	NI
Assenato (1987)	Italy	No (18)	(18)	(2.2)	NI	(40)
		Yes (18)	(61)	(7.9)	NI	(41)

PbB: blood lead, LPbS: log-e transformed semen lead. NI = No information available. [a]Measurements in seminal fluid (median).

variables important for the participants. By measuring both components of accumulation more thoroughly, the contribution of age was less important.

Percentage of time lived in Mexico City has served to distinguish between the accumulation of Pb because of age from that of living in a polluted environment (Brown et al. 2000). In this case, too few men had lived outside the City, and even less for a considerable amount of time (more than 80% of the men had lived 75% or more of their lives in Mexico City), in order for the percentage of time lived in Mexico City to be a discriminatory variable. Contrary to what Brown et al. found, in our final tibia model, percentage of life lived in Mexico City was not related to Pb after controling for age, although in the final patella model, it was. Limitations of this study include its cross-sectional design and lack of information about other body Pb predictors that might add to the knowledge of important sources and determinants, such as calcium, the intake of other micronutrients and exercise.

Conclusion

Lead levels found in this study are similar to those found in non-occupationally exposed men elsewhere, much lower than those found in occupationally exposed men and lower than the levels that have been associated with male fertility problems. The results of our analysis of blood and bone Pb determinants are mostly consistent with findings in other populations and reveal an ever decreasing roll of environmental exposures in body Pb burden. The inclusion of blood Pb as a predictor of semen Pb in the same regression model as patella Pb proved not to be adequate, since blood Pb is an intermediate step in the mobilization of Pb from bone to semen. Interestingly, endogenous Pb seems to be a significant exposure source in men, even though they do not have periods of high calcium demand that might trigger bone Pb release, as women do during pregnancy, lactation and menopause, and their bone turnover is not as intense as it is in growing children or teenagers.

Semen results provide useful information on the compartmental biodynamics of Pb in men. In the bivariate analysis, semen Pb predictors were very similar to those of blood, and the correlation between blood and semen Pb was relatively high, indicating the importance of exogenous sources in the Pb burden delivered to testes. Semen Pb was not affected by the frequency of sexual relations, which could be considered a means of excretion. Our results also suggest that this metal is significantly mobilized from trabecular bone and readily delivered from blood to testes, indicating the importance of an endogenous source.

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