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ORIGINAL ARTICLE

The influence of lead on the biomechanical properties of bone tissue in rats

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Abstract

Introduction and objective. Environmental lead (Pb) is a serious public health problem. At high levels, Pb is devastating to almost all organs. On the other hand, it is difficult to determine a safe level of exposure to Pb. More than 90% of the Pb in the adult human body and 70% in a child's body is stored in the bones. In the presented study, the effects of lead exposure on bones were studied for rats treated orally with Pb acetate in drinking water for 14 days. The hypothesis was tested that lead exposure negatively affects bone structure.

Materials and methods. Femur strength was measured in a three-point bending test, whereas infrared spectroscopy (FTIR) was used to measure molecular structural changes.

Results. Lead significantly decreased the ratio of area of two types of vibrational transitions, which are highly specific to mineral to matrix ratio. The results of the biomechanical study show that femurs of rats treated by Pb-acetate appeared to be weaker than bones of the control group, and may produce a condition for the development of higher risk of fractures. Additionally, a great difference in body mass was observed between control and the Pb acetate-treated groups.

Conclusions. The lower bone mineral content and the weaker mechanical properties of bones from Pb-treated rats are associated with the pathologic state dependent of the exposure of lead.

Key words

lead, FTIR, rat, mechanical properties of bones

INTRODUCTION

Lead exposure is an important public health problem, especially in the urban environment [1], and even a low-dose is hazardous [2]. Lead-contaminated dust and lead-based paints are the main sources of lead poisoning. However, there are many other sources, including: ceramic glazes, electronic waste, cosmetics, toys, water pipes, solder in canned food and lead from soils [3, 4]. Clinical and science studies have suggested that lead is devastating to the human body. Lead poisoning accounts for about 0.6% of the global burden of disease [5]. Lead enters the human body from the environment by inhalation and through the digestive system. Even small amounts of lead are accumulated in the kidneys, liver, brain, lungs and muscles. However, 95% of lead in the body is deposited in the bones [6, 7]. Accumulation of lead in the skeleton begins during foetal development and continues throughout adulthood [8]. From calcified tissue, Pb is released slowly, depending on bone turnover rates. According to Rabinowitz et al. [9], the elimination half-life of Pb in cortical bone is approximately 10–30 years. The retention and absorption of Pb appear to be greater in children and infants than in adults. Numerous studies have demonstrated that lead is transferred from the mother to the foetus, and showed that elevated blood levels of Pb in pregnant woman can cause premature birth, low birth weight, foetal malformation, and subsequent developmental delays in the infants [10, 11, 12]. The most sensitive targets for lead toxicity are the nervous system, the haematological systems, and the kidneys. Exposure to high amounts of lead resulting in a high level in the blood (>4.8 μmol/l) can cause acute toxic encephalopathy [13].

The aim of the presented study was evaluation of the changes in the bone tissue in rats intoxicated with lead acetate. To determine the possibility of bones quality reduction by Pb, two studies were conducted: biomechanical strength assay and FTIR spectroscopy measurement.

MATERIALS AND METHOD

All of the experiments were carried out on N=16 male Wistar rats sexually mature (three months) from a laboratory farm in Rembertów (Warsaw, Poland) divided into two groups, as follows: (C) N=8 control rats and (Pb) N=8 lead acetate-treated rats. The study rats were kept in the same animal room under constant temperature (22 °C). Food and water were freely available in the home cages. Animals from the study group were intraperitoneally injected with lead acetate. An aqueous solution of lead acetate (15 mg/kg body weight) was administered to rats once daily for 14 consecutive days. The control rats received *aqua pro* injection in the constant volume of 0.5 ml/100g body weight. On the 24th day the animals were decapitated. The same femur was used for biomechanical and spectroscopy measurements. Studied bones were dissected, cleaned of soft tissue and kept at

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–15 °C. All experimental procedures were approved by the Local Ethics Committee at the Medical University in Lublin, Poland.

Fourier-transform infrared spectra were recorded with Nicolet 6700 FTIR spectrometer from the Thermo Scientific Company (Waltham, MA, USA). To record the FTIR spectrum of each sample, 1 mg of the powder of rat femoral head was mixed with 200mg of KBr and compressed into a pellet for FTIR analysis. The femurs were dried before FTIR measurements. The spectra were obtained in the range 400–4000 cm^{-1}, with a frequency resolution of 4 cm^{-1} in the transmission mode. For each sample, 16 scans were accumulated, Fourier transformed and averaged. Background spectra were collected under the identical condition for each sample. Bone ashes were recorded at the same conditions as described previously. Data analysis and deconvolution of FTIR spectra were carried out with GRAMS software (Galactic Industries Corporation, Salem, NH, USA). The amid region, phosphate and carbonate region of the FTIR spectrum of rat femoral head were fitted with both Gaussian and Gaussian-Lorentzian component bands. The accuracy of the component band frequency determination was higher than 0.1 cm^{-1}. The sample was reduced to ash in a muffle furnace. Bone lead and bone control were ashed for 24 h at 637 °C. Ash mass and FTIR spectra were recorded.

The whole bone biomechanical parameters were measured with the 3-point bending strength test using Lloyd LRX tensile testing machine (Lloyd Instruments, Bognor Regis, West Sussex, UK), as described previously [14]. Bones were frozen and stored at –15 °C. The femurs were thawed at room temperature 12 hours before the mechanical test. Individual femurs were placed in a customized holder with the span between supports fixed at 2 cm, and the crosshead lowered at a constant speed of 2 mm/min until the femur fractured. The maximum load (F_{max}) that fractures the femoral shaft and deflection corresponding to the maximum force (l_{max}) were generated from the load-displacement curves (Fig. 1. The stiffness of the bone shaft (H) was defined as the slope of a linear region of the load-displacement curve. Additionally,

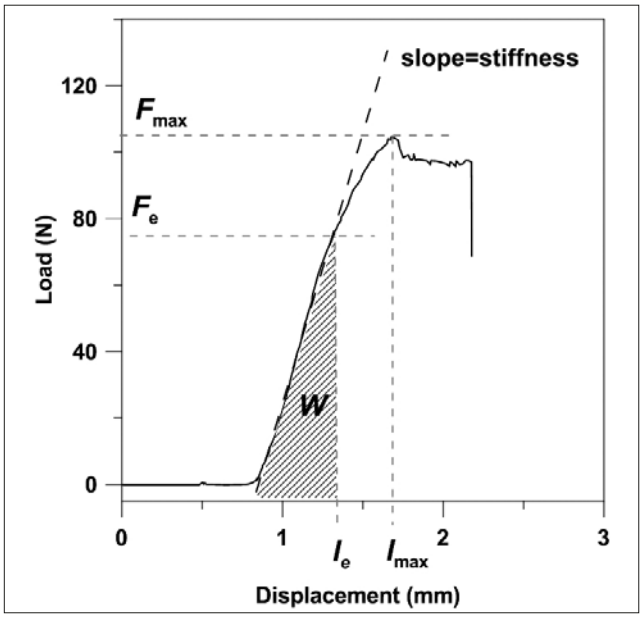

Figure 1. A typical force-displacement curve of the rat femur with indication of respective parameters

the force at the limit of elasticity (F_e), the deflection of femur at the limit of elasticity (l_e), and the energy stored in the bone without resulting in its permanent deformation (W), modulus of elasticity (E) were determined.

Statistical analysis. FTIR spectral parameters and biomechanical parameters are reported as mean ± S.D (standard deviation). Statistical significance was determined using the Student's Test where a p values equal to or less than 0.05 were accepted as significantly different from the control group.

RESULTS

Initial body mass did not differ significantly between control and Pb acetate-treated animals. Consequently, body mass of rats was determined after 0, 7 and 14 days of lead acetate administration. Table 1 shows that Pb treatment significantly affected body mass. The greatest difference in body mass was observed during 7-day and 14-day of treatment. Experimental rats lost about 5% of their initial body mass during that period. The final weight measurement was conducted on the last day (10 days after last lead acetate administration). A clear difference was observed between the control and experimental group. The control rats increased body mass by about 39% while the Pb-treated rats only about 5%. In the presented study, a significant decrease (p=0.0107) was also observed in the density of dry mass of bones, which was determined after 24 hours bone heating at 105 °C temperature. The density of dry mass of Pb-exposed bone was 11% lower than the dry density of control bone. The transverse diameter (d_t) and conjugate diameter (d_c) of femur in the plane parallel to the loading force were also different between the control and Pb acetate-treated group. Both femoral external diameters of rats treated by Pb-acetate (d_t=4.35±0.30mm and d_c=4.02±0.19mm) were smaller than those of the normal rats (d_t=3.57±0.50mm and d_c =3.08±0.17mm).

Table 1. Increases in body weight of animals from experimental (E) and control (C) groups

day of experiment	C		E		significance levels (p)
	body weight [g]	standard deviation (SD)	body weight [g]	standard deviation (SD)	
0	225	4.6	222	2.9	p=0.6986
7	256	5.9	211	3.6	p<0.001
14	277	9.0	212	9.0	p=0.0002
19	305	12	228	15	p=0.0012
24	312	8.5	235	5.6	p<0.0001

Infrared spectroscopy was used to measure molecular changes in the Pb acetate-treated bones. FTIR spectra of the processed rat femoral head samples are shown in Fig. 2. Two bands were examined to obtain information about the inorganic and organic components of rat femoral head. Integrated area of the PO_4^{3-} phosphate stretching peak and area of the protein C=0 stretching (amid I) peak were calculating to determine the relative ratio of mineral to matrix phase (Fig. 3) [15]. The mineral to matrix ratio is indicative of the relative quantity of inorganic components in bones and

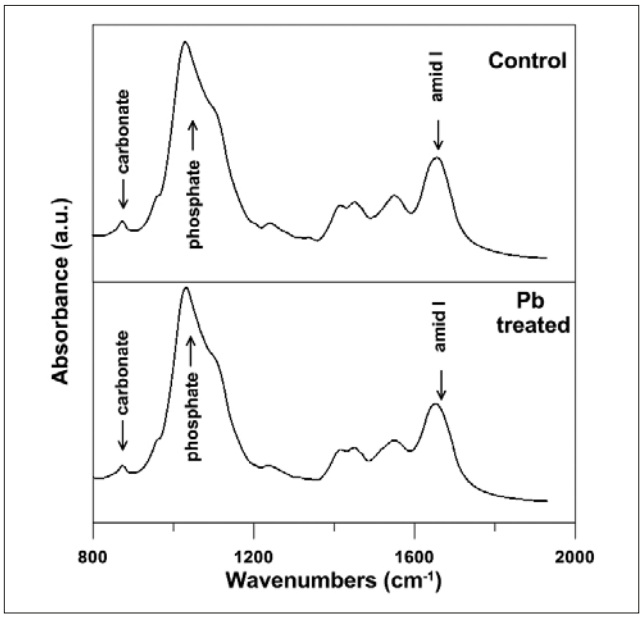

Figure 2. FTIR spectra of rat femoral head obtained from the control and lead-treated rat.

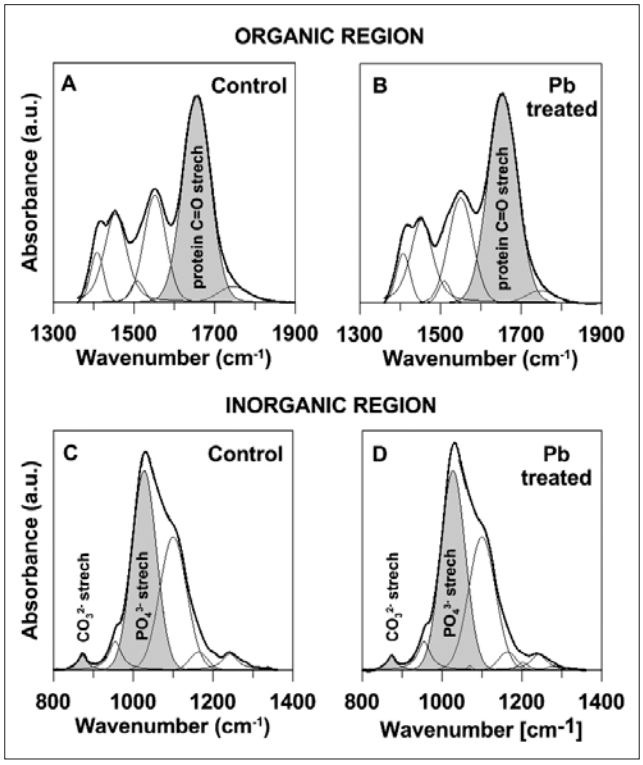

Figure 3. Curve-fitting analysis of the FTIR spectra for the control (A, C) and lead-treated (B, D) bones with the Gaussian components. Raw spectrum (thick solid line), curve-fitting spectrum (dashed line)

is related to the ash content of studied femurs (Fig. 4). Pb significantly (p=0.01) decreased the mineral to matrix ratio. These findings are in agreement with the results reported in [16]. The area of the CO_2^{-3} peak and the PO_4^{3-} phosphate stretching peak were calculating to determine the relative carbonate content which plays a significant role in bone resorption. In contrast to the level of mineralization, lead exposure did not affect the relative carbonate substitution into mineral lattice. This result was confirmed by the analysis

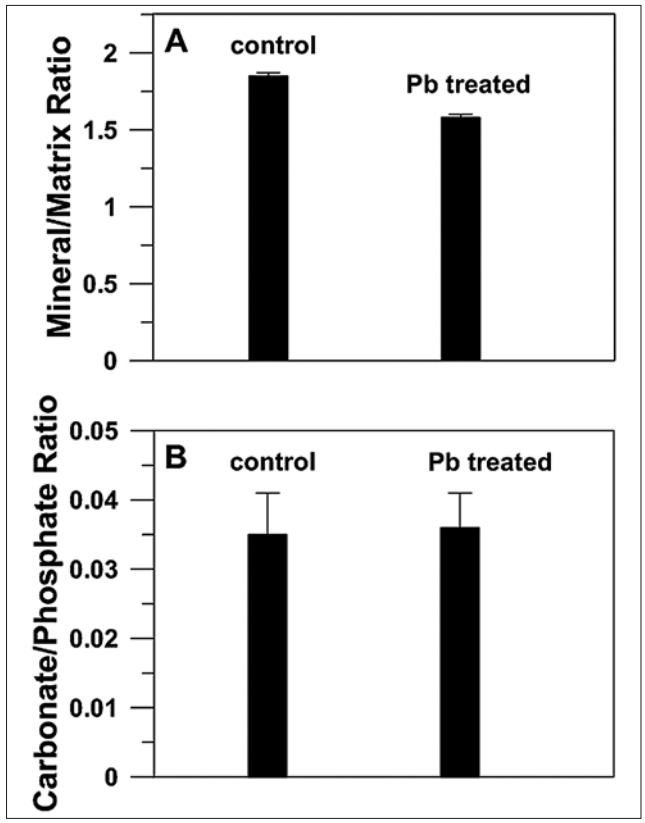

Figure 4. Plots of mineral to matrix ratio (A) and carbonate to phosphate ratio (B) for control and Pb acetate treated bones. Values are reported as mean ± SD. Significance is indicated p=0.01 in the case of mineral to matrix ratio and p=0.06 in the case of carbonate to phosphate ratio

of FTIR spectrum of bone ash. The presented results indicate that the ash of rat femoral heads from Pb-exposed rats exhibited the same level of carbonate substitution in the hydroxyapatite crystal as control group. The ratio of band 1,460 cm^{-1} to band 1,040cm^{-1}, respectively, was associated with the carbonate band with the phosphate group, was not significantly different in both groups. However, a significant difference ($p<0.0001$) was found in ash mass for control (255±6 mg) and lead treated bone (183±3mg).

In order to determine the relation of bone mineralization to differences in bone strength, femurs were tested in bending measurement. The biomechanical parameters determined for both groups are shown in Tab. 2. In Pb acetate-treated rats, significant decreases were observed in maximum load

Table 2. Mechanical parameters of femoral shafts (values expressed as mean±S.D) and significance levels between control group (C) and experimental group (E)

parameter	C		E		significance levels (p)
	mean	standard deviation (SD)	mean	standard deviation (SD)	
F_{max} [N]	104	12	72	21	p=0.0025
F_e [N]	88	13	65	14	p=0.0040
I_{max} [mm]	0.70	0.05	0.80	0.08	p=0.0096
I_e [mm]	0.49	0.06	0.51	0.06	p=0.5190
W [mJ]	17.6	4.3	13.5	3.4	p=0.0516
H [N/mm]	226	11	168	25	p=0.0459
E [MPa]	46.0	4.5	40.2	3.3	p=0.0107

(–30%), force at the limit of elasticity (–26%), stiffness (–26%) and modulus of elasticity (13%) in comparison to the control group. Ronis et al. [17] also demonstrated the reduction in bone strength in Pb-treated rats. The biomechanical properties of bones include their stiffness and strength and elasticity are determined by its microarchitecture, its geometry (shape, size) and the thickness of the cortical layer. Under loading conditions, the possible fractures occur on the shaft. It was found that the thickness of the femoral shaft was significantly smaller in rats which had been Pb acetate-treated for 14 weeks, than that in the control rats.

DISCUSSION

FTIR technique is a known powerful tool for diagnosing bone disease that alters calcified tissue and provides information on the average chemical composition, including collagen phase and mineral structure changes of the sample [18, 19]. In the presented study, differences have been shown in calcified tissue composition between lead-treated and control bones. Lead significantly decreased the mineral to matrix ratio in lead-intoxicated bones. In addition, the observed biomechanical differences, as measured by three-point bending test, suggest that mineral to matrix ratio is one of the determinants of bone strength.

Osteoporosis is a disease that causes weakness of the bone, characterized by low bone mass associated with the high risk of fractures [20]. Several studies report that increased lead exposure is associated with a decrease in bone mineral density [21, 22]. Presented in this study, Pb-exposure results suggest loss of the bone inorganic components, which give bone its strength. Lower bone density, lower bone mineral content and weaker mechanical properties of bone in lead-treated rats, seem to be associated with the pathologic state dependent of the exposure of lead acetate.

In fact, the probable mechanisms of the Pb toxicity in bone is the sum of several processes [23]. In brief, the Pb may directly or indirectly alter regulation of hormones, which modulate bone cell function, particularly 1,25-dihydroxyvitamin D3 [24]. Additionally, Pb impairment of bone matrix production was also reported [25]. Finally, the Pb has high affinity for the typical calcium-binding sites and may substitute calcium, magnesium, and phosphorus content. Mineralization of bone, which is essential for its strength, is a complex process in which apatite crystals are placed in the matrix. Our presented results showing a decrease in bone density and reduction of mineral to matrix ratio indicate a defective mineralization process in lead-exposed rats.

CONCLUSION

In conclusion, the presented results indicate that Pb has a determinant impact in bones which is manifested by biochemical, structural and biomechanical lesions. Moreover, they indicate an absolute necessity to look for factors and methods to protect the organism from the accumulation of lead in the bones. Finally, because of rapid industrialization, much works needs to be done to the reduction of exposure to lead. This can be achieved, among other things, by understanding that exposure to environmental lead is serious public health hazard.

REFERENCES

1. Tong S, Schirnding YE, Prapamontol T. Environmental lead exposure: a public health problem of global dimension. Bulletin of WHO. 2000; 78 (9): 1068–1077.
2. Grandjean P. Even low-dose lead exposure is hazardous. The Lancet 2010; 376 (9744): 855–856.
3. Chuturkova R, Iossifova Y, Clark S. Decrease in Ambient Air Lead Concentrations in Varna, Bulgaria, Associated with the Introduction of Unleaded Gasoline Ann Agric Environ Med. 2010; 17: 259–261.
4. Vargha B, Ötvös E, Tuba Z. Investigations on ecological effects of heavy metal pollution in Hungary by Moss-Dwelling water bears (Tardigrada), as bioindicators. Ann Agric Environ Med. 2002; 9: 141–146.
5. WHO. Global health risks: mortality and burden of disease attributable to selected major risk. Geneva, World Health Organization. 2009.
6. Barry PSI. A comparison of concentrations of lead in human tissues. Br J Ind Med. 1975; 32 (2): 119–139.
7. Rabinowitz MB, Wetherill GW, Kopple JD. Kinetic analysis of lead metabolism in healthy human. Clin Invest. 1976; 58 (2): 260–270.
8. Silbergeld EK. Lead in bone: implications for toxicology during pregnancy and lactation. Environmental Health Perspectives 1991; 91: 63–70.
9. Rabinowitz MB. Toxicokinetics of bone lead. Environ Health Perspect. 1991; 91: 33–37.
10. Gardella C. Lead Exposure in Pregnancy: A Review of the Literature and Argument for Routine Prenatal Screening. MD Obstetrical & Gynecological Survey 2000; 56 (4): 231–238.
11. Markowitz ME, Saenger P, Bijur P. Inverse correlation with growth velocity in lead toxic children. Pediatric Res. 1990; 27: 62–67.
12. Szkup-Jabłońska M, Karakiewicz B, Grochans E, Jurczak A, Nowak-Starz G, Rotter I, Prokopowicz A. Effects of blood lead and cadmium levels on the functioning of children with behavior disorders in the family environment. Annals of Agricultural and Environmental Medicine. 2012; 19 (2): 241–246.
13. Canfield RL, Henderson CRJr., Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentration below 10 μg per deciliter. N Engl J Med. 2003; 348: 1517–1526.
14. Olchowik G, Chadej-Polberg E, Tomaszewski M, Polberg M, Tomaszewska M. The influence of caffeine on the biomechanical properties of bone tissue during pregnancy in a population of rats. Folia Histochem Cytobiol. 2011; 49(3): 504–511.
15. Palaniappan PL. RM, Krishnakumar N, Vadivelu M, Vijayasundaram V. The study of the changes in the biochemical and mineral contents of bones of Catla. Environmental Toxicology. 2010: 25(1): 61–67.
16. Monir AU, Gundberg CM, Yagerman SE, van der Meulen MC, Budell WC, Boskey AL, Dowd TL. The effect of lead on bone mineral properties from female adult C57/BL6 mice. Bone. 2010; 47(5): 888–894.
17. Ronis MJJ, Aronson J, Gao GG, Hogue W, Skinner RA, Badger TM, Lumpkin ChK. Skeletal effects of developmental lead exposure in rats. Toxicological Sciences 2001; 62: 321–329.
18. Carden A, Morris MD. Application of vibrational spectroscopy to the study of mineralized tissues. J Biomed Opt. 2000; 5: 259–268.
19. Boskey A, Mendelsohn R. Infrared analysis of bone in health and disease. J Biomed Opt. 2005; 10 (3): 031102–031106.
20. Riggs BL, Melton LJ. The worldwide problem of osteoporosis: insights afforded by epidemiology. Bone 1995; 17 (5): 505–511.
21. Gruber HE, Gonick HC, Khalil-Manesh F, Sanchez TV, Motsinger S, Meyer M, Sharp CF. Osteopenia induced by long-term, low- and high-level exposure of the adult rat to lead. Miner Electrolyte Metab. 1997; 23 (2): 65–73.
22. Escribano A, Revilla M, Hernandez ER, Seco C, Gonzalez-Riola J, Villa LF, Rico H. Effect of lead on bone development and bone mass: a morphometric, densitometric, and histomorphometric study in growing rats. Calcif Tissue Int. 1997; 6 (2): 200–203.
23. Pounds JG, Long GJ, Rosen JF. Cellular and Molecular Toxicity of Lead in Bone. Environmental Health Perspectives.1991; 91:17–32.
24. Rosen JF, Chesney RW, Hamstra A, DeLuca HF, Mahaffey KR. Reduction in 1,25,-dihydroxyvitamin D in children with increased lead absorption. N. Engl. J. Med. 1980; 302 (20): 1128–1131.
25. Hass GM, Landerholm W, Hemmens A. Inhibitionof intercellular matrix synthesis during ingestion of inorganic lead. Am J Pathol. 1967; 50: 815–819.