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Lead and Calcium Content in the Human Hip Joint

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Abstract Concentration of lead in bone, unlike in soft tissues, increases during the lifetime and reflects severity of exposure to this element. The main aim of the study was to determine concentrations of lead and calcium and to find possible relationship between calcium and lead in the tissues of the hip joints obtained from inhabitants of the Upper Silesian Industrial Area. We also attempted to identify factors that might affect this relationship. The samples were harvested intraoperatively during total hip replacement procedures; in most cases, the indication for the surgery was hip osteoarthritis. Concentrations of lead and calcium were measured with a Pye Unicam SP-9 acetylene-oxygen flame atomic absorption spectrometer. The highest mean concentration of lead was found in the cancellous bone from the femoral head, followed by articular cartilage, cortical bone and the intertrochanteric cancellous bone (0.75 μg/g). The smallest concentration was found in the joint capsule (0.19 μg/g). The highest mean concentration of calcium was found in cancellous bone from the femoral head, followed by cancellous bone from the intertrochanteric area, cortical bone, articular cartilage and joint capsule. The concentration of lead showed no correlation with sex. The bone concentration of calcium decreased with age. In the analysed hips, this finding was true in the cortical bone, as well as in the cancellous bone of the intertrochanteric area. Statistically significant correlation between calcium and lead was found only in the hip articular cartilage.

Keywords Lead · Calcium · Human hip joint

Introduction

The lead absorbed into body is mostly (90%) accumulated in bone [1]. The conditions associated with increased bone turnover, such as pregnancy, lactation and menopause, result in greater liberation of this element from skeletal stores into the bloodstream [2, 3].

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The lead, due to its chemokinetic properties in skeleton, is involved in processes related to osteoporosis. In the settings of physiologic calcium release related to bone turnover, the stored lead may be incorporated into the bone instead of calcium. Evidence from numerous in vivo and in vitro experiments suggests that lead affects function of osteoblasts and osteoclasts in both direct and indirect ways. On one hand, lead influences renal function, leading to impaired conversion of vitamin D into active metabolites, which in turn results in decreased plasma levels of the active 1,25-dihydroxycholecalciferol and the parathormone (PTH). On the other hand, lead directly interferes with activity of osteoblasts and osteoclasts by disturbing hormonal signalling of these cells [1, 3–6].

Lead blocks all subtypes of calcium channels. It has been found that drugs affecting calcium channels exert a similar effect on the influx of lead into cells [7–9]. The competition for ion channels is probably one of the mechanisms of interaction between lead and calcium in the intestines; the less calcium ions in the diet, the more lead ions are absorbed. Additionally, lead ions compete with calcium for calcium-binding proteins [9–11].

Accumulation of lead varies between the different parts of the skeleton and depends on bone type (cortical or cancellous). Wittmers et al. [12] found that the concentration of lead during growth and until the age of 35 years was higher in vertebrae than in tibias. After skeletal growth has been completed, lead is stored mostly in the compact cortical bone, e.g. in tibia. Lead is deposited in areas of the most active bone metabolism. In cancellous bone, comprising 20% of skeleton, which has faster turnover, the excretion half-life is usually shorter than in the cortical bone, comprising 80% of skeleton, which has lower metabolic rate. This difference in half-life time ranges from a few years in vertebrae to more than 16 years in the calcaneum (os calcis). The half-life excretion time of lead stored in cortical bone was calculated at 5 to 15 years, but may be as high as 25 years [13–15].

The aim of the study was to investigate the concentration of lead and calcium in the human hip joint, to calculate the ratio between their concentrations and to find any possible relationship between these elements.

Material and Methods

The study was performed on tissue samples harvested from human hip joints belonging to inhabitants of the Upper Silesian Industrial Area, Poland. The samples were obtained intraoperatively during total hip replacement procedures performed in the Community Hospital in Siemianowice Śląskie. In total, we harvested samples from 53 patients (43 females and 10 males). The average age of patients in the study group was 65.6 years (67.2 years for women and 58.4 for men). Nine subjects admitted smoking habit; the remaining 44 individuals denied tobacco use. In most cases, the indication for surgery was primary hip osteoarthritis.

In the study group, there was only environmental, not the occupational, exposure to lead. However some of the subjects lived for 20 or 30 years in the Upper Silesian Industrial Area, where environmental exposure is high due to concentration of heavy industry. The lead content in particulate matter (PM10) in the air between years 2000 and 2008 was shown in Fig. 1 [16].

The study was approved by the local ethics committee (decision no. NN-6501-160/I/06 issued on November 21, 2006). The patients' informed consent was not required because the study was conducted on material which is considered a medical waste and normally would be disposed of after the surgery.

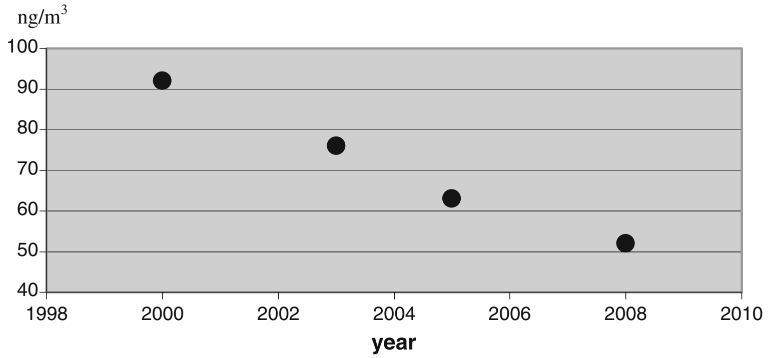

Fig. 1 Concentration of lead in particulate matter (PM10) in the air. Note that the lead content decreased over the period of 8 years (2000–2008) [17]

Further analysis was conducted on the following samples:

1. Femoral head excised in situ
2. Anterolateral aspect of the joint capsule, which was routinely excised in order to open the hip joint during surgery
3. A box-shaped fragment of cancellous bone from the intertrochanteric area (this fragment was routinely chiselled out from the femoral bone in order to create a starting point for preparation of the proximal femur before implantation of the prosthetic stem)

The femoral heads were debrided from residual soft tissues. Fragments of joint capsule, ligament of the head of the femur, and femoral neck (especially its medial cortex) were removed with various instruments, such as Liston bone cutting forceps, Luer bone rongeur and bone curette. In the next stage, we used bone curette and Luer rongeur to remove articular cartilage and then the subchondral bone until we obtained a rounded "core" made of the cancellous bone only. In some patients, the cartilage was absent or scarce due to progression of osteoarthritis. The subchondral layer of bone had various widths: from virtually non-existent to fraction of millimetre to a good few millimetres of dense, sclerotic bone in advanced hip osteoarthritis. The remaining part of the femoral head consisted mostly of the cancellous bone, sometimes with heterogeneous microarchitecture, with areas of osteosclerosis and subchondral cysts (geodes) filled with a fibrous connective tissue. The harvested samples were placed in polyethylene bags, labelled and stored in a freezer at temperature of −20±1°C. Laboratory equipment used for chemical analysis of samples was thoroughly cleaned with chromic acid, then with 20% HNO_3 (V) (Merck, Germany) and double distilled water, and finally it was dried in a hermetic dryer. The samples were initially dried at a temperature of 100±2°C and then incinerated at a temperature of 420±2°C in a hermetic muffle oven. A known mass of ash (usually 2.00 g) was solubilised with 2 ml of Supra pure 65% HNO_3 (V) (Merck, Germany). The resulting solution was transferred into a 25-ml volumetric flask and made up to the 25 ml mark with double-distilled water.

The samples were examined at the Department of Toxicology, Medical University of Silesia and in a certified laboratory of the Department of Environmental Monitoring of the Central Mining Institute (site number AB 145 in the registry of Polish Centre of Accreditation). Concentrations of lead, calcium and other elements were measured with a Pye Unicam SP-9 acetylene-oxygen flame atomic absorption spectrometer.

In order to verify the accuracy of the measurements, samples taken from four randomly chosen femoral heads were tested both at the Department of Toxicology, Medical University of Silesia and in the Department of Environmental Monitoring of the Central Mining Institute. The average concentrations of particular metal elements were calculated based on the results of six test runs for each sample and differed by 3.7% (lead). The procedure was further validated by measurement of lead and calcium concentration in reference materials NIST-1400. The differences between the measured and the reference values ranged from 2.6% to 6.3% for lead and from 5.1% to 10.8% for calcium. The detection limits of the method were 0.013 μg/ml (lead) and 0.080 μg/ml (calcium).

The statistical analysis of the gathered data was performed with the Statistica PL v. 7.0 software. The possible relationship between variables was described with the Pearson's correlation coefficients ($p<0.05$). The *U* Mann–Whitney nonparametric test was used for statistical analysis.

Results

The lead content in the sampled parts of the hip joints, harvested from patients living in areas of various concentration of particulate matter in the air, ranged from 0.06 to 40.98 μg/kg. There were significant differences in the average lead concentration between the particular components of the studied hip joints. The highest average concentration of lead was found in cancellous bone from the femoral head (1.32 μg/g), followed by articular cartilage (1.25 μg/g), cortical bone (1.05 μg/g) and samples of the intertrochanteric cancellous bone (0.75 μg/g). The smallest concentration was found in the joint capsule (0.19 μg/g).

The coefficient of variability for lead was calculated at 223%. The analysis of lead content in various parts of the hip joint revealed that there were differences between particular components of the joint and between sexes (Table 1). The most significant differences between males and females were found in the articular cartilage ($p=0.0047$). In cancellous bone, cortical bone and cancellous bone from the intertrochanteric area differences in lead content were not statistically significant ($p=0.6$). On the other hand, the lead concentrations in the tissues comprising the joint capsule were identical for both sexes.

The research into calcium concentration in the hip joint showed a high dispersion of results, which ranged from 0.53 to 184.4 mg/g. The highest mean concentration of calcium was found in cancellous bone from the femoral head (160.04 mg/g) followed by cancellous bone from the intertrochanteric area (42.63 mg/g), cortical bone (39.46 mg/g), articular cartilage (21.62 mg/g) and joint capsule (3.04 mg/g).

The coefficient of variability of calcium calculated for concentration of calcium in the hip joint was 98% and was similar in both male and female patients (Table 2). The statistically significant differences in calcium concentration between males and females were found in the articular cartilage and in the cancellous bone from the intertrochanteric area ($p<0.001$).

The results for the concentration of lead showed roughly normal distribution, whereas concentration of calcium showed a negative skew and higher "peakedness" as compared to the normal (Gaussian) distribution. We found that concentration of lead in the hip joint tended to increase with age. However, the correlation was not statistically significant and the "*r*" coefficient was very low. The statistically significant correlation between concentrations of lead and calcium was found only in the articular cartilage ($r=0.59$)—Fig. 2.

The calcium content in bone decreased with age. In case of the hip joint, it was seen in the cortical bone, as well as in the intertrochanteric cancellous bone. By contrast,

Table 1 Summary statistics for concentration of lead in the hip joint [microgrammes per gramme of dry weight]

Sampled tissue	Arithmetic mean	Standard deviation	Geometric mean	Range		Confidence interval for mean		Percentile rank				Coefficient		
				Min.	Max.			10	50	90	95	Excess	Kurtosis	Variation %
Females (n=43)														
Articular cartilage (n=43)	3.28	8.05	1.24	0.29	40.98	0.32	6.23	0.35	1.00	3.98	22.81	4.14	17.66	246
Cancellous bone (n=43)	1.75	1.61	1.31	0.34	9.08	1.26	2.25	0.56	1.22	3.27	5.01	2.70	9.69	92
Cortical bone (n=43)	1.62	1.64	1.00	0.08	7.50	0.98	2.25	0.11	1.39	4.60	4.82	2.22	5.71	101
Samples of cancellous bone from the intertrochanteric region (n=43)	1.18	1.92	0.68	0.16	9.59	0.40	1.95	0.21	0.67	1.80	4.58	3.83	15.81	163
Joint capsule (n=43)	0.25	0.26	0.18	0.06	1.18	0.17	0.34	0.08	0.16	0.75	0.96	2.26	4.67	101
Males (n=10)														
Articular cartilage (n=10)	1.98	1.94	1.31	0.26	6.22	0.36	3.60	0.26	1.45	6.22	6.22	1.75	3.41	98
Cancellous bone (n=10)	1.97	1.73	1.38	0.46	5.26	0.80	3.13	0.50	1.34	4.64	5.26	1.09	−0.27	88
Cortical bone (n=10)	1.93	0.06	0.46	5.60	0.46	3.92	1.38	1.29	5.60	5.60	1.90	1.92	3.89	3
Samples of cancellous bone from the intertrochanteric region (n=10)	1.25	0.69	1.10	0.51	2.52	0.61	1.89	0.51	1.13	2.52	2.52	1.01	0.93	55
Joint capsule (n=10)	0.25	0.24	0.19	0.07	0.84	0.07	0.43	0.07	0.18	0.84	0.84	2.41	6.31	94

Table 2 Summary statistics for concentration of calcium in the hip joint [milligrammes per gramme of dry weight]

Sampled tissue	Arithmetic mean	Standard deviation	Geometric mean	Range		Confidence Interval for mean		Percentile rank				Coefficient		
				Min.	Max.			10	50	90	95	Excess	Kurtosis	Variation %
Females (n=43)														
Articular cartilage (n=43)	27.81	19.75	22.03	5.25	90.84	20.56	35.05	8.25	19.43	54.59	62.75	1.37	2.03	71
Cancellous bone (n=43)	160.73	13.19	160.15	108.21	184.40	156.67	164.79	149.18	161.53	176.05	178.63	−1.33	4.94	8
Cortical bone (n=43)	48.97	20.35	37.64	1.24	83,614.64	41.08	56.86	3.26	5.03	73.73	81.93	−0.96	1.40	42
Samples of cancellous bone from the intertrochanteric region (n=43)	44.85	16.65	41.30	7.52	79.64	38.12	51.57	28.81	41.01	75.47	75.76	0.41	0.42	37
Joint capsule (n=43)	4.61	5.31	3.07	0.53	31.71	2.89	6.33	0.94	3.51	8.50	12.53	3.77	18.04	115
Males (n=10)														
Articular cartilage (n=10)	25.48	18.60	20.13	5.07	64.13	9.93	41.03	5.07	19.69	64.13	64.13	1.43	2.22	73
Cancellous bone (n=10)	160.12	13.02	159.60	128.54	177.98	151.37	168.87	147.86	161.34	172.52	177.98	−1.40	3.14	8
Cortical bone (n=10)	49.61	42.52	41.30	61.09	41.30	56.70	49.24	48.43	61.09	61.09	6.76	0.88	1.28	86
Samples of cancellous bone from the intertrochanteric region (n=10)	51.81	20.76	47.97	22.96	86.88	32.61	71.00	22.96	53.67	86.88	86.88	0.33	0.55	40
Joint capsule (n=10)	4.49	5.90	2.90	0.90	19.92	−0.05	9.02	0.90	2.70	19.92	19.92	2.79	8.04	131

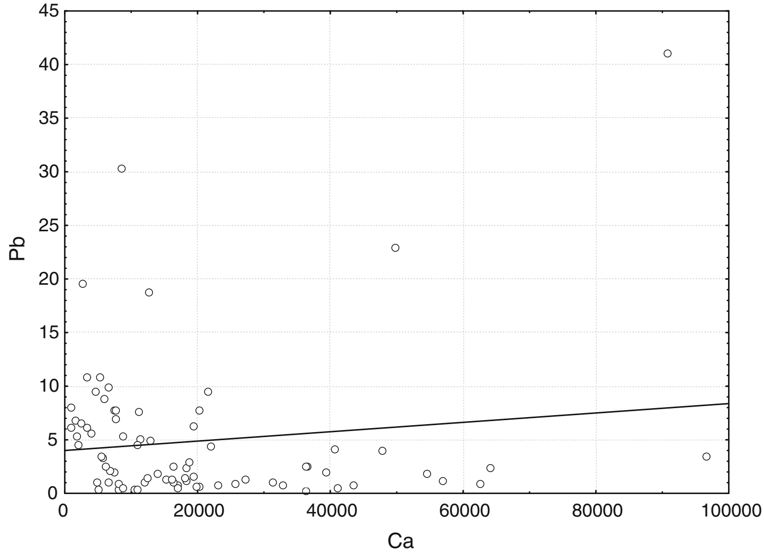

Fig. 2 Correlation between concentration of calcium and lead in the articular cartilage harvested from the femoral head

concentration of calcium in the joint capsule increased with age; it could be assumed that over time, the capsule undergoes degenerative changes leading to accumulation of calcium deposits. In all parts of the hip joint, the concentration of calcium differed significantly between the age groups (see Fig. 3).

We found statistically significant differences in concentration of lead in the articular cartilage between the patients aged 50 to 60 and 71 to 80 years. There were also statistically significant differences in concentration of lead in the cortical bone between the groups aged

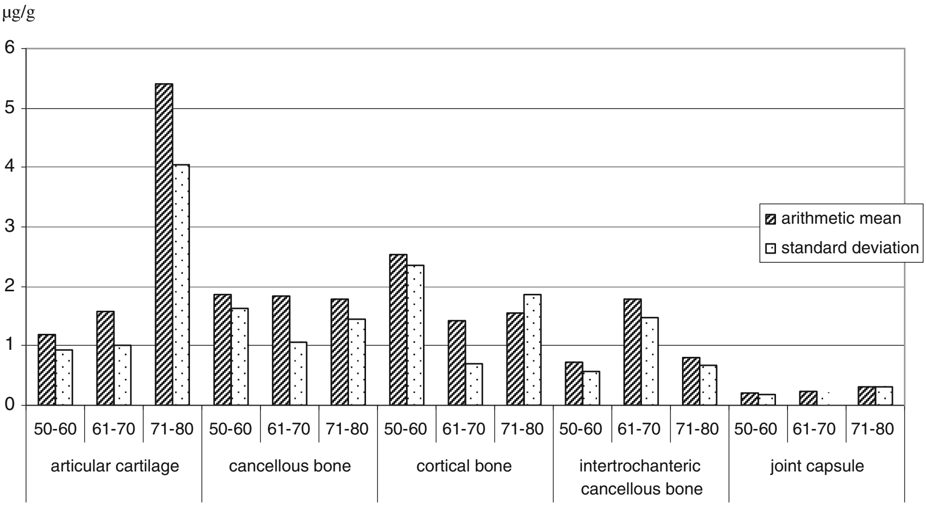

Fig. 3 The arithmetic mean and standard deviation lead concentration in different parts of the hip joint, stratified by age

50 to 60 and 61 to 70 years and between those aged 50 to 60 and 71 to 80 years. In the cancellous bone femoral head, the statistically significant differences in concentration of lead were found between the patients aged 50 to 60 and 61 to 70 years, as well as between those aged 61 to 70 and 71 to 80 years (see Fig. 4).

There were significant differences in Pb/Ca ratio in particular parts of the hip joint, i.e. the cancellous bone from the femoral head, the cortical bone, joint capsule and the intertrochanteric cancellous bone. The highest ratio was found in the joint capsule (0.62×10^{-4}) and the lowest in the cancellous bone femoral head (0.08×10^{-4}). The ratio in the intertrochanteric cancellous bone was similar to that calculated for the cortical bone (0.18×10^{-4} and 0.26×10^{-4}, respectively) (see Fig. 5). The Pb/Ca ratio, calculated for the whole femoral head, was similar for male and female patients (0.28×10^{-4} vs. 0.26×10^{-4}). The differences between males and females in the selected parts of the hip joint were not statistically significant (p=0.4).

We also carried out a principal component analysis including the following variables: the part of the hip joint, the patient's age, sex, place of residence, smoking habit, and concentration of lead and calcium. In the whole study population, we found four important factors whose values were greater than 1, and their total variance was 70.5%. The first factor indicates that the type of tissue (from which the individual parts are built of) and calcium content have the most important role in the structure of the hip joint. The second factor indicates the role of gender and its inversely proportional correlation with age. The third factor underlines the impact of the place of residence. The last (fourth) factor indicates the importance of lead content (see Table 3).

Discussion

Due to difficulties with obtaining a representative number of samples for chemical analysis, bone is rarely used as a research material in toxicology studies. There are very few reports on concentration of trace elements in bone fragments obtained during surgical procedures.

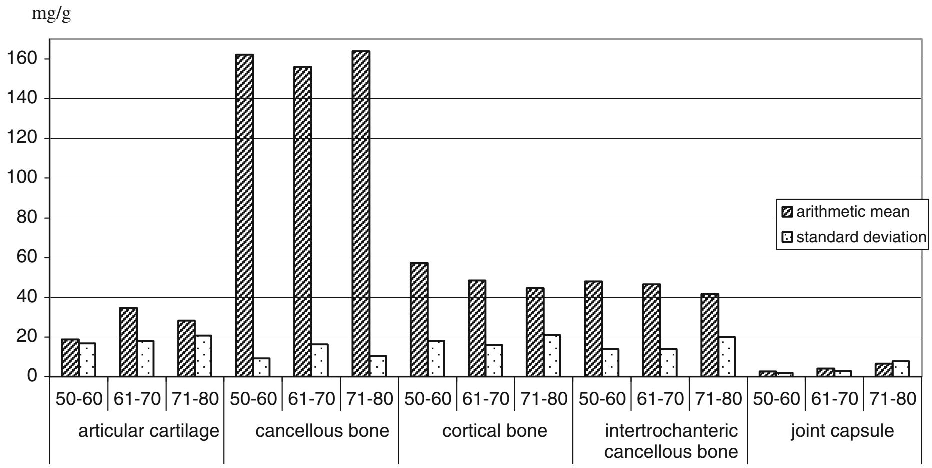

Fig. 4 The arithmetic mean and standard deviation calcium concentration in different parts of the hip joint, stratified by age ($p<0.001$)

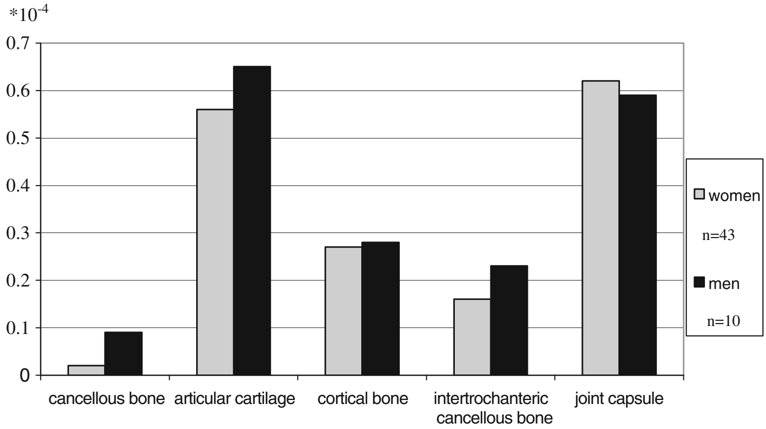

Fig. 5 The value of Pb/Ca ratio in particular components of the hip joint

Therefore, previous studies were mostly performed on bones found during archaeological excavations or from samples taken from deceased individuals [12, 17–20].

Comparison of the various parts of the hip joint indicates that the cancellous bone has the highest concentration of the investigated elements, whereas the lowest concentration was found in fragments of the joint capsule. This finding was attributed to fundamental differences in the microscopic anatomy of these two tissues and confirmed that bone has greater capacity to accumulate metals than the connective tissue.

The average lead concentration in the investigated parts of the hip joint strongly points out to the fact that the aforementioned element accumulates mostly in the cancellous bone, which is the part with the highest metabolic activity. The pattern of lead concentration in bone supports the thesis that this metal is stored mostly in the areas with the greatest metabolic activity. Such pattern was noted by Wittmers et al. [12, 17] and Kuo et al. [18]. The published data show that lead concentration in bone is seven times higher than in the connective tissue, which is the core element of the joint capsule. Similar differences were also reported for calcium level, but in this case, the concentration in bone was as much as 53 times higher.

The presence of lead in bone comprising the hip joint has an anthropogenic nature, which is confirmed by the high coefficient of variation. The highest variability was found in

Table 3 Results of principal component analysis for the hip joint

	Components			
Parameter	1	2	3	4
Part of hip joint	**0.79**	−0.12	−0.12	0.19
Sex	0.07	**0.75**	0.22	0.31
Age	−0.14	**−0.79**	0.02	0.05
Place of living	−0.03	0.06	**0.80**	0.07
Smoking habit	−0.02	0.41	−0.59	−0.23
Pb	−0.54	−0.06	−0.28	**0.76**
Ca	**−0.76**	0.13	0.08	−0.32

the articular cartilage, which might be explained by the fact that the cartilage is the outermost layer of the femoral head; hence, it has constant and most intensive contact with a synovial fluid.

The conducted search for possible relationship between lead and calcium in various parts of the hip joint showed no correlation between bone concentrations of these elements, except for the articular cartilage where it was statistically significant. This observation justifies the opinion that the impact of lead on concentration of calcium in the hip joint remains unclear. By contrast, in the case of calcium, we found a synergistic interaction with chromium and zinc and an antagonistic interaction with cadmium and iron. A correlative analysis revealed a correlation between levels of calcium and lead in the articular cartilage (r=0.59) irrespectively of gender.

Comparison between the results of our study and the already published data showed that lead level measured in ribs of Japanese people was similar to lead concentration in our samples [19, 20], although calcium content in all parts of the hip joint was much lower and comparable to the aforementioned studies only in case of the cancellous bone. By contrast, in Taiwanese population, concentration of lead in bones was higher (7 μg/g). Calcium content was also higher than in our study (82 vs. 63 mg/g) [18]. Our results are also much lower than findings on bones found at excavation sites. The authors, however, presume that this was caused by contamination of the excavated bones with lead present in soil [19, 20]. The bone lead content changes with age. This statement can be confirmed by the publication of Kuo et al. [18] who showed that the highest concentration of lead was found in the age group of 80 years (9.36 μg/g) and the lowest in people aged under 40 years (4.51 μg/g).

In our study group, the lead content increased markedly with age in the samples of the articular cartilage and the joint capsule. In the cortical bone, lead concentration was highest in the group of the youngest patients, whereas in the intertrochanteric cancellous bone, the highest concentration was found in individuals aged 61 to 70 years. On the contrary, in the cancellous bone harvested from femoral head, lead content was similar in all age groups.

Calcium is the most important element in the chemical content of bone, whereas lead has a significant role as a calcium antagonist, as it may substitute calcium in hydroxyapatite. Therefore, a ratio between bone levels of lead and calcium (Pb/Ca ratio) has been frequently calculated. The Pb/Ca ratios calculated in our study (0.08×10^{-4} to 0.62×10^{-4}) were much higher than those published in the study by Ericson et al. [21] (1.6×10^{-7} to 73×10^{-7}) and higher by one order of magnitude than those of Hisanaga et al. [19] (0.2×10^{-6} to 5.2×10^{-6}).

Conclusions

The highest accumulation of lead was found in the cancellous bone. The lowest concentration of lead and calcium was found in the joint capsule, which should be attributed to a different microscopic anatomy of this tissue. The level of lead and calcium changes with age. However, these changes are selective; the concentration of lead increases with age in articular cartilage and joint capsule, whereas the concentration of calcium decreases with age in cortical bone and the intertrochanteric cancellous bone.

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