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Relative Changes of Elements in Human Osseous Tissue

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The role of elements in the human osseous and disease has been extensively studied in recent years. Elements are classified into two groups: essential and trace elements. Some trace elements are essential to health, but some trace elements are potentially toxic. An excess or decrease of certain trace elements can be detrimental to health. Information on elements of human bones and of their body burden is useful for assessing nutrition and for prevention and control of various disease states caused by mineral or trace element imbalance (Saltman et al. 1990). For example: barium, aluminum and chromium do not show any significant changes in their concentrations within one era. The average concentration of calcium changes from 0,2 g/g to 0,25 g/g in ash, and the concentration of phosphorus from 0,11 g/g to 0,13 g/g in ash. The concentration of lead in the Edo era was 7-10 times higher than it was in the earlier periods. In the Edo era, the considerable increase of the content quotients of Pb/Ca, Pb/Al, Al/Fe and Pb/Mn was observed. This can be explained by the presence of lead in food as a result of using lead utensils or by emission of pollutants during primitive technology of melting that was used at that time. The occurrence of elements in bones coming from the excavations can be the measure of the physiological level of these metals in bones, but there is a possibility of modification of the real metal contents in bones considering their various contents in soil and their secondary migration to bones.

Using ASA method (Kuo H.W. et al. 2000), a sequence of elements: Al, Ba, Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr, Ti and Zn in ribs coming from Japanese population were indicated. As a result, high contents of sodium and magnesium have been noticed.

The content of zinc was higher than 100 μg/g. Where as the concentrations of strontium, potassium, aluminium and iron were higher than 10 μg/g. While the contents of lead and chromium had the value of 1μg/g, the concentrations of cadmium, cobalt, copper and nickel were lower than 1μg/g (Yoshinaga et al. 1989). In the case of iron and lead, significant differences related to sex were noted. Among male these elements occurred in higher concentration.

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MATERIALS AND METHODS

In order to estimate the physiological level of metals in the osseous tissue, numerous bones coming from excavations (Akira et al. 1988; Ericson et al. 1979; Grendjean et al. 1979, Koshugi et al. 1989) were studied. The studies made it possible to reconstruct the diet and the level of environmental pollution. The contents of such metals as: Sr, Mg, Ba, Zn, Cu and Fe in bones depend on the diet, whereas the occurrence of lead depends on environmental pollution (Kwapuliński et al. 1995; Kwapuliński et al. 2001).

The contents of various metals: Al, Ba, Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Na, Ni, P, Pb, Sr, Ti, V and Zn were analyzed in ribs coming from the excavations in Japan (Koshugi et al. 1986). Fossil bones dated back to a few pre-historic eras and historic periods :Jomon (5000-2300 B.C.), Yoyoi (2300-1700 B.C.), Kofun (1700-1400 B.C.), Kamakura & Muromachi (700-500 B.C.), and Edo (300-120 B.C.).

The indication of the basic composition and the content of element series was made by the sequence X-ray fluorescence spectrometer with wave - length dispersion. An X-ray lamp with a double positive plate and maximum power of 3000 W was used as an excitation source. The matrix error was minimalized by using correction factor during the preparation of standardization curves.
While choosing standards for the X-ray fluorescent spectrometry, the possibility of chemical composition variety of the analyzed biological samples was taken into account. The results showed that analytical standardization curves, in possibly wide range of concentration, include full range of changes of the analyzed elements in the samples. To determine the precision and accuracy of the method, a sample of an average percentage of composition was X-rayed 10 times in order to indicate its chemical composition. The established values are the measure of precision, whereas the measure of accuracy are enumerated confidence limits at the significance level of α=0,95.

The delectability limits of the contents of particular elements in ash by means of XRF were the following: 2,8 μgAs/g, 4,6 μgBa/g, 0,8 μgCd/g, 1,8 μgCo/g, 9,5μgCr/g, 2,8 μgCu/g, 5,9 μgMn/g, 2,9 μgNi/g, 1,3 μgPb/g, 4,6 μgRb/g, 3,4 μgSr/g, 0,5 μgV/g, 2,7 μgZn/g, 1,6 μgZr/g.

The indication of contents of the following elements: Ni, Mn, Cr, Cd, Pb, Cu, Fe, Zn, Mg, K, Na, Ca was done by means of Atomic Spectrophotometric Absorption Method, using PYE Unicam SP- 9 apparatus in the acetylene- air flame. The delectability of particular metals was the following: 2 μgCd/dm^3, 3 μgCr/dm^3, 1 μgCu/dm^3, 10 μgFe/dm^3, 2 μgNi/dm^3, 2 μgMn/ dm^3, 1 μgZn/dm^3.
The subject of the study were samples of femur capitulum with various degeneration of a hip joint. Heads of femur were obtained inter-operationally, in the process of endoprosthesisplasty.

The separate group made up the heads of femoral bone obtained from the Bank of Tissues, from persons of unknown origin during the autopsy. These samples were radioactively sterilized and lyophilized. In addition, the head of femur was divided into cortex, spongy bone and articular surface in aseptic conditions. The heads of femoral bone deformation mainly consisted in flattening and fungoid bone outgrowths witch appeared mostly neighboring the head and the femoral neck. There was also the defect of joint surface mainly in cotyle roof, as well as in the upper-external part of the head of femoral bone.
The samples of the head of femoral bone came from people inhabiting the area of Cracow, Zielona Góra and Silesian towns such as Katowice, Siemianowice, Mysłowice and Czeladź.

The studied population consisted of samples coming from 26 female and 11 male individuals. The age range of this population was 54 - 86 years. The average age was 69,2 years: 67,1 for female and 73,2 for male. There were 20 non-smokers (among them 18 female and 2 male).

RESULTS AND DISCUSSION

The analysis of Al, Fe, Pb, Ca corpse contents was made in Venezuela (Granadillo et al. 1992). No disorders in bone metabolism had been noted among these people during their lifetime.
Our data suggest that the removal of calcium from bones may take place as a results of the influence of the other metals, which are extraneous to bone tissue, in mineral bone structure. As a consequence, the mentioned metals may lead to bone decalcification. The order of including shows the following sequence: Pb, Al, Fe, V.

Understanding metabolic processes which are connected with the bone tissue pathology or result from eating habits of a given historical period requires the analysis of contents changes of the chosen metals on the one hand, and the qualification of the content quotients of chemio-derived elements on the other. In particular, it refers to Ca, Fe, Cd. The comparison of average contents of the chosen metals in bones coming from the excavations dating from 5000 - 120 B.C. with those dating from 1999-2000 is presented in table 1. In the pre-historic period, the contents of Pb, Fe, Co, Mn increased significantly in the years of 300 - 120 B.C. Nowadays, the average lead content in bones decreased by 8 μg/g what makes 1/4 of the value of the Edo era.

Contemporary, the content of Cd in bones is comparable with that of the Edo era; it is only higher by about 0,5 μg/g. In the Edo era, the structural change in methods of obtaining metals for objects of daily use caused the increase to about 1100 μgFe/g, and then the decrease to the level of 162±229,80 μgFe/g.

Next, the pre-historic period was compared with 1999-2000 years. The comparison was based on the following quotients: Pb/Ca, Ba/Ca, Ca/P, Fe/Al,

Table 1.The comparison of average metal contents in bones coming from the excavations with our samples from 1999- 2000 [μg/g].

Elements	Jomon 5000-2300 p.n.e.	Yayoi 2300-1700 p.n.e.	Kofun 1700-1400 p.n.e.	Kamakura Muromachi 700-500 p.n.e.	Edo 300-120 p.n.e.	Our results 1999-2000
Ca [mg/g]	256	238	257	254	204	36,5
P [mg/g]	126	112	129	123	106	42,4
Al [μg/g]	1800	1627	1385	1024	1069	395
Ba [μg/g]	35	27	29	27	34	13
Cd [μg/g]	4,8	7,0	9,4	4,5	1,8	0,3
Co [μg/g]	0,65	0,85	1,06	0,94	2,53	3,0
Cr [μg/g]	22	28	22	24	19	4,4
Cu [μg/g]	38	36	27	35	38	12,7
Fe [μg/g]	930	1415	104	4020	10970	162
K [μg/g]	180	276	107	170	209	2190
Mg [μg/g]	760	1301	1422	1153	1003	1720
Mn [μg/g]	103	232	224	131	1167	0,26
Na [μg/g]	2552	2922	2967	3224	3478	17058
Ni [μg/h]	1,5	4,6	1,3	2,1	3,5	1,2
Pb [μg/g]	1,2	1,3	1,9	1,7	12,2	4,0
Sr [μg/g]	560	538	298	884	335	282
Ti [μg/g]	46	42	49	38	66	720
V [μg/g]	22	24	24	31	39	5
Zn [μg/g]	217	183	362	259	271	200

Sr/Zn, Pb/Al, Pb/Fe, Pb/Mn table 2. For example, the coefficient Ba/Ca nowadays decreased by $2x10^3$ times. This coefficient value changed from 0,136 in 5000-2300 B.C. to 0,166 in 300-120 B.C. Nowadays, the proportion of Ca/P increased by about 4 times in relation to the prehistoric period and the Fe/Al quotient increased by seven times.

Table 2. The comparison of quotients related to the metal contents from the pre-historic period and from 1999-2000.

Quotient	Jomon	Yayoi	Kofun	Kamakura& Muromachi	Edo	Japanese Ribs	Our Results
Pb/Ca	0,0046	0,0054	0,007	0,0066	0,059	0,014	$0{,}0098\times 10^{-3}$
Ba/Ca	0,136	0,113	0,112	0,106	0,166	-	$0{,}36\times10^{-4}$
Ca/P	2,032	2,125	1,992	2,065	1,925	1,943	8,54
Fe/Al.	0,516	0,869	0,075	3,929	10,262	0,761	3,62
Sr/Zn	2,581	2,939	0,823	3,413	1,236	0,607	1,003
Pb/Al.	0,00066	0,0008	0,00137	0,00166	0,01141	0,079	0,009
Pb/Fe	0,0012	0,0009	0,0182	0,0004	0,0011	0,104	0,0025
Pb/Mn	0,011	0,005	0,008	0,013	0,0104	-	0,0596

The influence of the industrial and economic development on the occurrence of Al, Pb Fe, Mn is proved by the fact that Pb/Al quotient is by 13 times, Pb/Fe by 2,5 times, Pb/Mn by 5,4 times, Ca/Pb by 4 times and Fe/Al by 7 times higher. At present, the proportion of Sr/Zn contents decreased by 7 times in relation to the pre-historic period. Cultural differences regarding eating habits, kinds of utensils are shown in the reduction of Pb/Ca contents by 2 thousand times, Ba/Ca by about $2{,}6\times10^4$ (tab. 2.). The results suggest that during the bone decalcification, we can range the engaging of the metals in the following way: Pb, Fe, Al and Mn.

The appointed range differs in this matter that it contains vanadium which substitute for manganese.
Koshugi determined molar factors indicated in bones coming from the excavations. In table 3, there are molar factors estimated for Japanese ribs and the studied population of inhabitants of the chosen Polish towns. The comparison of the molar factors of the analyzed elements shows the decreasing tendency for the quotients of Pb/Ca, Sr/Zn and Pb/Al contents.

Historical growth after about 2300 years was stated for the quotient of Fe/Al contents - by about 3,5 times, Pb/Mn - by about 30 times

The analysis of these results indicates the significance of eating habits in a given historic period.

Table 3. The relation of molar factors of some elements.

Quotient	Jomon	Yayoi	Kofun	Kamakura & Muromachi	Edo	Japanese Ribs	Our results
Ca/Pb	1.57	1.66	1.54	1.59	1.47	1.5	6.6
Fe/Al.	0.31	0.44	0.42	2.94	6.96	0.234	1.74
Sr/Zn	2.15	2.63	0.73	3.30	1.49	0.45	0.75
Pb/Ca	0.0010	0.0010	0.0015	0.0014	0.0131	0.0027	0.0019×10^{-3}
Pb/Al.	0.0002	0.0002	0.0003	0.0004	0.0021	0.0102	0.0012
Pb/Fe	0.0006	0.0007	0.0006	0.0002	0.0016	0.0280	0.0006
Pb/Mn	0.0051	0.0084	0.0076	0.0084	0.0185	-	0.1582

The relation of Sr/Zn, which now amounts to 0,75 in comparison to pre-historic periods, when it was 2,15-3,30, is a meaningful example. It results from lower supply of strontium and higher supply of zinc, what is typical for the present diet rich in proteins. This view is confirmed by the work of Klepinger (Klepinger et al. 1984). The growth in relations of Pb/Ca, Pb/Al, Pb/Mn indirectly indicates the degree of these elements, which come from the soil.

In order to complete the above considerations, the following content quotients were estimated: Ag/Cu, Sr/Cu, Zn/Cu, Cd/Cu, Mo/Cu, Mn/Cu, Fe/Cu, Zn/Ag, Co/Sr, Ni/Zn, Cd/Zn, Fe/Cd, Co/Cd, Mn/Fe, Mn/Mo, Mn/Ni, Mn/Pb.

Acording to literature references, the relations of Mn/Fe, Mn/Mo, Mn/Ni between the considered quotients refer to synergic reactions, whereas the remaining quotients are typical for antagonistic relation.

For the first time, such a wide range of evaluation was done and it may be treated as a reference system to historical comparisons, and contemporary tendencies of interaction changes.

For example, the antagonisms of Zn/Cu, Cd/Cu, Co/Cd are three times stronger. The relations between Cd/Zn maintain at the same level. It proves that there is a competitive influence between these elements. The synergic phenomena between Mn/Ni and Mn/ Pb are much less intensive.

Table 4. The quotients which characterize the types of interaction between metals as a criteria of environmental exposure evaluation.

Quotient	Jomon	Yayoi	Kofun	Kamakura&Muromachi	Edo	Japanese Ribs	Our Results
Ag/Cu	-	-	-	-	-	-	0.1106
Sr/Cu	14.737	14.944	11.037	25.257	8.815	366.956	15.574
Zn/Cu	5.710	5.083	13.407	7.40	7.131	604.348	15.524
Cd/Cu	0.126	0.194	0.348	0.128	0.047	0.391	0.387
Mo/Cu	-	-	-	-	-	-	0.221
Mn/Cu	2.710	6.444	8.296	3.742	30.710	-	0.332
Fe/Cu	24.473	39.305	3.852	114.857	288.684	135.652	79.1
Zn/Ag	-	-	-	-	-	-	140.335
Co/Sr	0.0012	0.0015	0.0035	0.001	0.0075	0.0014	0.011
Ni/Zn	0.007	0.025	0.003	0.008	0.013	0.003	0.007
Cd/Zn	0.022	0.038	0.025	0.017	0.006	0.00065	0.025
Fe/Cd	193.75	202.14	11.06	893.33	6094.44	346.666	204.3
Co/Cd	0.135	0.121	0.112	0.208	1.405	1.333	0.428
Mn/Fe	0.110	0.164	2.154	0.032	0.106	-	0.004
Mn/Mo	-	-	-	-	-	-	1.5
Mn/Ni	68.666	50.434	172.308	62.381	333.428	-	3.0
Mn/Pb	85.833	178.46	117.894	77.058	95.655	-	1.676

In many cases, the comparison of data referring to rib bone points out many differences in content levels of the chosen elements. Both the results of our research and the pre-historic data let us conclude that the relation of Sr/Cu contents is 24 times higher in the case of ribs.

At the same time, the quotient of Co/Sr contents is 10 times higher in case of femur capitulum. It was also stated that in the case of the femoral head, the content quotient of Ni/Zn is 2 times higher, and the content quotient of Cd/Zn is 40 times higher. Furthermore, in the case of femoral bone the content quotients of Fe/Cd and Co/Cd are about 3 times lower.

The estimated quotients may constitute one of the more important criteria evaluating bone tissue usefulness as a potential biomarker of environmental exposure.

The results of the estimation may not only confirm typical kinds of elements interactions, but they also prove modern views on the subject, even in historical relation.

The estimated values of particular content quotients may be treated as a reference system for estimation of the future evaluation of environmental and professional exposure.

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