NEUTRON ACTIVATION ANALYSIS OF TRACE ELEMENT CONTENTS IN THE BONE SAMPLES OF HUMAN ILIAC CREST

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Introduction

The physicochemical properties of the apatite phase in bone are affected by trace element incorporation into the bone tissue.[1] It is the reason why deficiency or excess of F, Sr, Mn, Fe, and some other trace elements, is one of the factors which determines the biomechanical properties of bones.[2] Thus, chemical element analysis of bone tissue expands the knowledge of etiology of bone diseases, including osteoporosis, and may be applied for diagnostic, therapeutic and preventive purposes. Furthermore, bone tissue has been proposed as a dose monitor for the exposure of human body to bone-seeking elements,[3,4] and have been used as exposure monitors in occupational medicine and environmental health studies.[5] Moreover, a chemical element human bone analysis is often used in paleoanthropology for dietary and environment reconstruction, as well as in assessment of the social and economic status of human groups.[6] For the effective use of bone analysis in all the above-mentioned areas it is necessary to know normal levels as well as the age and gender changes of bone trace elements on the larger scale. There are several available studies describing trace elements analysis of bone tissue using chemical techniques and instrumental methods.[7-11] However, the majority of these data is based upon analysis of non-intact bones. In most cases, bone samples are treated with solvents in order to remove collagen, fat, marrow, and then are ashed and acid digested. There is evidence that some trace elements are lost or otherwise affected by these processes.[12-15] Instrumental neutron activation analysis (INAA) is a nondestructive method, which allows to investigate bone samples without harsh treatment and extensive preparation.

In the present study we analyzed the effect of age and gender on trace element mass fraction in human iliac crest. The fist objective was to use an intact bone sample, and the second objective was to use a bone commonly used for biopsies - iliac crest.

Experimental

Human iliac crest samples were collected at the Forensic Medicine Department of City Hospital (Obninsk). Anterior-superior parts from iliac crest (mainly from the right side) were obtained early after death at necropsy (within 24 hours) from 5 women and 24 men (age range 15 - 55 years). All subjects died suddenly due to automobile accident, falls, shootings, stabbing, hanging, acute alcohol poisoning, and freezing. None of those who died a sudden death had suffered from any systematic or chronic disorders before. Corpses were kept in a refrigerator at +2-4^{0}C. The bone specimens were immediately frozen at - 18°C until use. The sample sides contacted with surgical instruments were cut off and soft tissue and blood were removed. A titanium tool was used to cut and to scrub samples. Samples were freeze dried until constant mass was obtained. A titanium scalpel was used to cut thin longitudinal sections of the iliac crest weighing about 50-100 mg.

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins in the Institute of Physics, Georgian Academy of Sciences were used.[16] Corrected certified values of BSS element contents were reported by us before.[17] The iliac crest samples, ten CRM IAEA H-5 (Animal Bone) and SRM NIST 1486 (Bone Meal) sub-samples weighing about 50-100 mg, together with SSB tablets were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule. Reference material samples and standards were distributed among human bone samples along the whole ampoule length to allow correction of any neutron flux non-uniformity in the reactor channel. The quartz ampoule was soldered, positioned in a transport aluminum container and exposed to a 20-hour neutron irradiation in a vertical channel with a neutron flux of $1.7 \cdot 10^{12}$ $n \cdot cm^{-2} \cdot s^{-1}$. Seven days after irradiation samples were reweighed and repacked.

Each sample was measured twice, 7-10 and 40-60 days after irradiation. The second measurements were started 40 days after irradiation due to the high intensity of ^{32}P β-particles ($T_{1/2}$=14.3 d). To reduce this ^{32}P background, a combined lead-cadmium-copper-aluminum filter was used. The duration of the first and second measurements was 10 min and 10 hours, respectively. The gamma spectrometer included an HPGe detector (GEM 15180P, ORTEC, relative efficiency 15%) and a MCA combined with a PC (ASPRO-NUC) was used. The spectrometer provided a resolution of 1.8 keV on the ^{60}Co 1332 keV line. Processing of gamma spectra and computations of chemical element mass fractions in the examined samples were carried out using an ASPRO software package.[18]

A dedicated computer program of NAA mode optimization was used.[19] Using standard programs, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0,025 and 0,975 levels were calculated for trace element contents. The reliability of difference in the results between two age groups and between females and males was evaluated by Student's *t*-test.

Results

Table 1 depicts our data for 31 trace elements in ten sub-samples of CRM IAEA H-5 Animal Bone, and SRM NIST 1486 Bone Meal reference material, as well as the certified values of this material.

Table 2 represents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of 31 trace element contents in intact iliac crest of both females and males, taken together.

The comparison of our results with published data for trace element contents in the iliac crest of women and men is shown in Table 3.

Table 1. INAA data of trace elements of CRM IAEA H-5 Animal Bone and SRM NIST 1486 Bone Meal (mg/kg on dry weight basis)

Element	CRM IAEA H-5		This work results	SRM NIST 1486		This work results
	Mean	Type	Mean±SD	Mean	Type	Mean±SD
Ag	-	-	<0.02 DL	-	-	<0.02 DL
As	0.013	N	<0.1 DL	0.006	N	<0.1 DL
Au	-	-	<0.01 DL	-	-	<0.01 DL
Ba	72±28	C	<100 DL	-	-	270 DL
Br	3.6	N	<10 DL	-	-	<10 DL
Cd	0.023	-	<2 DL	0,003	N	<2 DL
Ce	-	-	≤0.03	-	-	≤0.02
Co	0.25	N	0.56±0.25	-	-	0.11±0.02
Cr	2.56	N	<0.8 DL	-	-	≤0.9
Cs	-	-	≤0.05	-	-	≤0.06
Eu	-	-	≤0.015	-	-	≤0.02
Fe	79±11	C	85±17	99±8	C	93±11
Gd	-	-	<0.25 DL	-	-	<0.25 DL
Hf	-	-	≤0.04	-	-	≤0.04
Hg	0.008	N	≤0.01	-	-	≤0.01
La	-	-	<0.05 DL	-	-	<0.05 DL
Lu	-	-	<0.003 DL	-	-	<0.003 DL
Nd	-	-	<0.1 DL	-	-	<0.1 DL
Rb	1.07	N	≤1.0	-	-	≤0.9
Sb	0.024	N	≤0.02	-	-	≤0.02
Sc	-	-	<0.001 DL	-	-	<0.001 DL
Se	0.054	N	≤0.05	0.13	N	≤0.05
Sm	-	-	<0.01 DL	-	-	<0.01 DL
Sr	96±17	C	103±9	264±7	C	263±10
Ta	-	-	≤0.08	-	-	≤0.08
Tb	-	-	<0.03 DL	-	-	<0.03 DL
Th	-	-	<0.05 DL	-	-	<0.05 DL
U	-	-	<0.07 DL	-	-	<0.07 DL
Yb	-	-	<0.03 DL	-	-	<0.03 DL
Zn	89±15	C	86±7	147±16	C	153±29
Zr	-	-	<0.2 DL	-	-	<0.2 DL

Abbreviations: Mean –arithmetical mean, SD – standard deviation
C - certified values, N - non-certified values, DL – detection limit

Table 2. Some statistical parameters of trace element contents in intact iliac crest of healthy females and males taken together (mg/kg on dry weight basis)

Element	Mean	SD	SEM	Min	Max	Median	P0.025	P0.975
Ag	<0.02 DL	-	-	-	-	-	-	-
As	<0.1 DL	-	-	-	-	-	-	-
Au	<0.01 DL	-	-	-	-	-	-	-
Ba	<100 DL	-	-	-	-	-	-	-
Br	<10 DL	-	-	-	-	-	-	-
Cd	<2 DL	-	-	-	-	-	-	-
Ce	<0.008 DL	-	-	-	-	-	-	-
Co	0.0073	0.0097	0.0024	0.00008	0.0420	0.0050	0.0011	0.0304
Cr	<0.8 DL	-	-	-	-	-	-	-
Cs	≤0.091	-	-	<0.002 DL	0.510	-	-	-
Eu	≤0.0045	-	-	<0.0001 DL	0.0290	-	-	-
Fe	177	129	24	13.5	477	153	22.2	455
Gd	<0.25 DL	-	-	-	-	-	-	-
Hf	<0.02 DL	-	-	-	-	-	-	-
Hg	≤0.0048	-	-	<0.0010 DL	0.011	-	-	-
La	<0.05 DL	-	-	-	-	-	-	-
Lu	<0.003 DL	-	-	-	-	-	-	-
Nd	<0.1 DL	-	-	-	-	-	-	-
Rb	1.89	0.79	0.22	0.97	3.40	1.80	0.98	3.31
Sb	≤0.04	-	-	<0.01 DL	0.12	-	-	-
Sc	≤0.0012	-	-	<0.0010 DL	0.0026	-	-	-
Se	≤0.112	-	-	<0.002 DL	0.260	-	-	-
Sm	<0.01 DL	-	-	-	-	-	-	-
Sr	225	146	27	36.5	770	187	43.1	499
Ta	<0.005 DL	-	-	-	-	-	-	-
Tb	<0.03 DL	-	-	-	-	-	-	-
Th	<0.05 DL	-	-	-	-	-	-	-
U	<0.07 DL	-	-	-	-	-	-	-
Yb	<0.03 DL	-	-	-	-	-	-	-
Zn	65.9	18.3	3.4	39.1	128	60.8	41.3	105
Zr	<0.2 DL	-	-	-	-	-	-	-

Abbreviations: Mean –arithmetical mean, SD – standard deviation, SEM – standard error of mean, min – minimal value, max – maximal value, P0.025 – percentile with 0.025 level, P0.975 – percentile with 0.975 level, DL – detection limit

To estimate the effect of age on the Co, Fe, Rb, Sr, and Zn content we examined two age groups: one comprised a younger group with ages from 15 to 35 years and the other comprised older people with ages ranging from 36 to 55 years (Table 4).

We used the entire dataset for both females and males taken separately, seeking to detect the presence of gender-related differences (see Table 5).

Table 3. Median, minimum and maximum value of means of trace element contents (mg/kg on dry weight basis) in human iliac crest according to data from the literature in comparison with our results

Element	Published data [Reference]			This work results
	Median of means [n]*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**	M±SD n=24
Ag	7.5 [1]	7.5 (1) [21]	7.5 (1) [21]	<0.02 DL
As	-	-	-	<0.1 DL
Au	-	-	-	<0.01 DL
Ba	71 [1]	71±16 (69) [22]	71±16 (69) [22]	<100 DL
Br	7.4 [1]	7.38±0.96 (69) [22]	7.38±0.96 (69) [22]	<10 DL
Cd	0.035 [1]	0.035 ±0.036 (10) [23]	0.035 ±0.036 (10) [23]	<2 DL
Ce	2.7 [1]	2.7±0.7 (69) [22]	2.7±0.7 (69) [22]	<0.008 DL
Co	0.35 [2]	0.046±0.037(20) [24]	0.52 (1) [22]	0.0073±0.0097
Cr	8.7 [2]	2.2±0.7 (10) [23]	15.1±5.8 (69) [22]	<0.8 DL
Cs	-	-	-	≤0.091
Eu	0.032 [1]	0.032±0.007 (69) [22]	0.032±0.007 (69) [22]	≤0.0045
Fe	263 [4]	183±78 (20) [24]	598±80 (69) [22]	177±129
Gd	-	-	-	<0.25 DL
Hf	0.12[1]	0.12±0.03 (69) [22]	0.12±0.03 (69) [22]	<0.02 DL
Hg	0.78 [1]	0.78±0.11 (69) [22]	0.78±0.11 (69) [22]	≤0.0048
La	0.56 [1]	0.56±0.08 (69) [22]	0.56±0.08 (69) [22]	<0.05 DL
Lu	-	-	-	<0.003 DL
Nd	-	-	-	<0.1 DL
Rb	-	-	-	1.89±0.79
Sb	0.25 [1]	0.25±0.04 (69) [22]	0.25±0.04 (69) [22]	≤0.04
Sc	0.08 [1]	0.08±0.01 (69) [22]	0.08±0.01 (69) [22]	≤0.0012
Se	0.30 [2]	0.13±0.04(20) [24]	0.47±0.08 (69) [22]	≤0.112
Sm	0.23 [1]	0.23±0.05 (69) [22]	0.23±0.05 (69) [22]	<0.01 DL
Sr	121 [3]	75 (1) 1984 [21]	162±8 (69) [22]	225±146
Ta	0.030 [1]	0.030±0.007 (69) [22]	0.030±0.007 (69) [22]	<0.005 DL
Tb	0.048 [1]	0.048±0.009 (69) [22]	0.048±0.009 (69) [22]	<0.03 DL
Th	0.31 [1]	0.31±0.06 (69) [22]	0.31±0.06 (69) [22]	<0.05 DL
U	-	-	-	<0.07 DL
Yb	-	-	-	<0.03 DL
Zn	151 [3]	150 (1) [21]	160±7 (69) [22]	65.9±18.3
Zr	-	-	-	<0.2 DL

Abbreviations: M - arithmetic mean, SD – standard deviation, [n]* – number of all references, (n)** - number of samples, DL – detection limit

Table 4. Effect of age on mean values (M±SEM) of trace element contents in intact iliac crest of healthy humans (mg/kg on dry weight basis)

Element	15-35 year n=14	36-55 year n=15	*p* t-test
Co	0.0102±0.0054	0.0050±0.0010	N.S.
Fe	156±33	199±35	N.S.
Rb	3.10±0.34	1.62±0.24	N.S.
Sr	213±32	237±44	N.S.
Zn	66.1±3.3	65.7±5.9	N.S.

Abbreviations: M - arithmetic mean, SEM – standard error of mean, N.S. - Not significant

Table 5. Effect of gender on mean values (M±SEM) of trace element contents in intact iliac crest of healthy humans (mg/kg on dry weight basis)

Element	Females n=5	Males n=14	*p* t-test
Co	0.0065±0.0011	0.0076±0.0035	N.S.
Fe	72.7±13.6	200±27	≤0.001
Rb	2.12±0.34	1.51±0.20	N.S.
Sr	307±28	208±31	≤0.05
Zn	66.7±6.3	65.8±3.9	N.S.

Abbreviations: M - arithmetic mean, SEM – standard error of mean, N.S. - Not significant

Discussion

Of 4 (Ba, Fe, Sr, Zn) and of 3 (Fe, Sr, Zn) trace elements with certified values for the CRM IAEA H-5 and the SRM NIST 1486 reference materials respectively, we determined contents of Fe, Sr, and Zn (Table 1). Mean values for these elements were in the range of 95% confidence interval. Mean values and 95% confidence intervals of Co, Cr, Hg, Rb, Sb, and Se were reported for CRM IAEA H-5, although those were not certified[20] (Table 1). Of these elements, a good agreement was obtained for Co, Hg, Rb, Sb and Se. Mean contents obtained for Cr appeared to be considerably lower than the appropriate values of Parr's report.[20] Good agreement with the certified data of CRM IAEA H-5 and SRM NIST 1486 reference materials indicate an acceptable accuracy of the results obtained in the study of trace elements of the iliac crest presented in Tables 2-5.

The mean values and all selected statistical parameters were calculated for 5 (Co, Fe, Rb, Sr and Zn) trace elements (Table 2). The contents of these trace elements were measured in all or a major portion of iliac crest samples. The contents of Cs, Eu, Hg, Sb, Sc, and Se were determined in a few samples of collection only. The upper limit of the mean values for the elements was found as the normalized sum of all individual contents and of the detection limits where the contents were not measured. The contents of Ag, As, Au, Ba, Br, Cd, Ce, Cr, Gd, Hf, La, Lu, Nd, Sm, Ta, Tb, Th, U, Yb, and Zr were lower than detection limits in all samples.

The obtained means for Fe, Sr and Zn, as shown in Table 3, agree well with values cited by other researches for the human iliac crest (including samples received from persons died from different diseases and bones from burials).[21-24] A number of values for chemical element mass fractions were not expressed on a dry weight basis by the authors of the cited references. However, we calculated these values using published data for water and ash contents in the cortical and trabecular bone[25] and our data for ratio of cortical and trabecular volumes of iliac crest samples. The Co means are an order of magnitude lower than the minimum mean value of previously reported data. This may be explained by contamination during either sampling or sample preparation when using instruments made of stainless steel. The upper limit of means for Se is nearly equal, and for Eu, Hg, Sb, and Sc are one-two orders of magnitude lower, than previously reported results (Table 3). The detection limits of INAA show that the contents of Ag, Ce, Cr, Hf, La, Sm, Ta and Th in iliac crest of health females and males are at least one-two orders of magnitude lower, than previously reported data (Table 3).

No statistically significant age variation was found for any of the studied trace elements (Table 4). It was shown that higher Sr mass fractions as well as lower Fe content were typical of female iliac crest compared with male iliac crest (Table 5). Low level Fe in the bone tissue of women compared with men can be attributed to physiological characteristics of the female body related to reproduction. A high content of Sr in the bones of women is likely due to differences in the ratio of nutrition foods of animal and plant origin. Usually, women consume more plant foods, which is the main supplier of Sr in the human body.

All the deceased were citizens of Obninsk, a small city of non-industrial region 105 km south-west from Moscow. None of those who died a sudden death had suffered from any systematic or chronic disorders before. Thus, our data for Co, Fe, Rb, Sr and Zn mass fractions in intact human iliac crest may serve as indicative normal values for residents of the Russian Central European region.

Conclusions

INAA using long-lived radionuclides allows determine the contents of 5 trace element (Co, Fe, Rb, Sr, and Zn) and upper limit of contents of other 6 trace elements (Cs, Eu, Hg, Sb, Sc, and Se) in intact iliac crest of healthy human. Mean values (M ± SEM) for mass fraction of Co, Fe, Rb, Sr, and Zn (mg/kg on dry weight basis) were: 0.0073±0.0024, 178±24, 1.76±0.24, 225±27, and 65.9±3.4, respectively. The contents of Ag, As, Au, Ba, Br, Cd, Ce, Cr, Gd, Hf, La, Lu, Nd, Sm, Ta, Tb, Th, U, Yb, and Zr are under detection limit of method.

No statistically significant age variation was found for any of the studied trace elements. It was shown that higher Sr mass fractions as well as lower Fe content were typical of female iliac crest compared with male iliac crest.

Our data for Co, Fe, Rb, Sr and Zn mass fractions in intact human iliac crest may serve as indicative normal values for residents of the Russian Central European region.

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