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The Effect of Age and Gender on 59 Trace-Element Contents in Human Rib Bone Investigated by Inductively Coupled Plasma Mass Spectrometry

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Abstract The effect of age and gender on 59 trace-element contents in rib bone of 80 apparently healthy 15–55-year-old women (n=38) and men (n=42) was investigated by inductively coupled plasma mass spectrometry. Mean values (M±SEM) for the mass fraction (milligrams per kilogram, on dry-weight basis) of Ba, Bi, Cd, Ce, Cu, Dy, Er, Gd, La, Li, Mn, Mo, Nd, Pb, Pr, Rb, Sm, Sr, Tb, Tl, U, Yb, and Zn for both female and male taken together were: Ba 2.5±0.2, Bi 0.015±0.002, Cd 0.044±0.005, Ce 0.029±0.002, Cu 1.05±0.06, Dy 0.0020±0.0003, Er 0.0011±0.0002, Gd 0.0015±0.0001, La 0.020±0.002, Li 0.040±0.002, Mn 0.354±0.004, Mo 0.052±0.006, Nd 0.011±0.001, Pb 2.24±0.14, Pr 0.0032±0.0004, Rb 1.51±0.06, Sm 0.0014±0.0001, Sr 291±20, Tb 0.00041±0.00005, Tl 0.00050±0.00003, U 0.0013±0.0001, Yb 0.00072±0.00007, and Zn 92.8±1.5, respectively. The upper limit of mean contents of Ag, Al, B, Be, Br, Cr, Cs, Hg, Ho, Lu, Ni, Sb, Te, Th, Ti, Tm, and Y were: Ag≤0.011, Al≤7.2, B≤0.65, Be≤0.0032, Br≤3.9, Cr≤0.25, Cs≤0.0077, Hg≤0.018, Ho≤0.00053, Lu≤0.00024, Ni≤1.05, Sb≤0.0096, Te≤0.0057, Th≤0.0030, Ti≤2.8, Tm≤0.00006, and Y≤0.0047, respectively. In all bone samples, the contents of As, Au, Co, Eu, Ga, Hf, Ir, Nb, Pd, Pt, Re, Rh, Sc, Se, Sn, Ta, V, W, and Zr were under detection limits. The Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb, and Yb contents increase with age. Higher Sr mass fraction is typical of female rib as compared to those in male bone.

Keywords Trace elements · Human rib bone · Age involution · Gender-related differences · ICP-MS

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Introduction

Osteoporosis is one of the most devastating and costly diseases to confront elderly women, and increasingly, men [1–3]. The incidence of bone diseases such as osteoporosis increases with advancing age, and fractures are usually the result of only relatively minor trauma. Osteoporotic fractures are a major cause of morbidity and mortality [4, 5]. Such fractures represent a significant social and medical problem in terms of treatment and rehabilitation [6–8].

Many of trace elements have a major effect on the bone condition and metabolism. Intake deficiency or excess of chemical elements causes debilitating bone diseases [9–11]. The list of inorganic nutrients or dietary contaminants which influence bone metabolism is gradually increasing as attention turns to some of less well-studied elements in the periodic table. Thus, to understand the role of trace elements in the etiology and pathogenesis of bone diseases including osteoporosis, it is necessary to determine the normal levels and age-related changes of bone trace elements in a large scale study.

There are several reviews and texts regarding chemical element analysis of different human bones, using chemical techniques and instrumental methods [12–16]. However, the majority of these data are based upon non-intact bones. In most cases, bone samples are ashed or are treated with solvents in order to remove collagen, fat, and marrow before they are ashed. There is evidence that some elements are lost by this process and their content ratio also being affected [17–19].

In the present study, ribs from subjects in the age range 15–55 years were analyzed with two objectives in mind. The first objective was to use intact bones and the second was to perform measurements on bones mostly affected by bone disease. Ribs will easily remodel (i.e., have high metabolic turnover rates), demonstrated by their sensitivity to diagnosis of change. Investigations have so far indicated that there is an association between effects of bone disorders and trabecular bone. Trabecular bone is the main structure of rib bone therefore ribs are considered a good model to detect bone abnormalities.

All studies were approved by the Forensic Medicine Department of Obninsk City Hospital and the Medical Radiological Research Center Ethical Committees.

Material and Methods

Samples of human rib bone were obtained at postmortem from intact cadavers (38 female and 42 male, 15–55 year old) within 24 h of death. The majority of samples were taken from the third, fourth, fifth, or sixth rib from the right side. The samples were immediately frozen at −18°C until use. Each death had resulted from automobile accidents, falls, shootings, stabbings, hanging, acute alcohol poisoning, or hypothermia.

A tool made of titanium and plastic was used to clean samples off soft tissues and blood. Samples were freeze dried until constant mass was obtained. A titanium scalpel was used to cut thin cross-sections of the rib weighing about 50–100 mg.

Rib bone samples were placed in one-chamber autoclaves (Ancon-AT2, Ltd., Russia) to which 1.5 mL of concentrated HNO3 (Nitric acid 65%, maximum 0.0000005% Hg, GR, ISO, Merck) and 0.3 mL of H2O2 (pure for analysis) were added and then heated for 3 h at 160–200°C for the samples to decompose. Then the autoclaves were cooled to room temperature. The solutions from the autoclaves were diluted with deionized water to 20 mL and transferred to plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without samples and the resultant solutions were used as control samples.

The contents of some major (Ca, K, Mg, Na, P, and S) and several trace elements (Al, B, Ba, Cu, Fe, Li, Mn, Sr, V, and Zn) in the obtained solutions were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). The results of these determinations were earlier reported [20].

Then the same solutions were used to determined the content of Ag, Al, As, Au, B, Ba, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Ga, Gd, Hf, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Re, Rh, Sb, Sc, Se, Sm, Sn, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb, Zn, and Zr by inductively coupled plasma mass spectrometry (ICP-MS) using the Spectrometer X-7 ICP-MS (Thermo Electron, USA).

The measurements were made at the following spectrometer parameters: RF generator power, 1,250 W; nebulizer, PolyCon; spray chamber, cooling 3°C; plasma gas flow rate, 12 L/min; auxiliary flow rate, 0.9 L/min; nebuliser flow rate, 0.9 L/min; sample update, 0.8 mL/min; resolution, 0.8 M.

The main parameters of mass-spectrum measurements were: detector mode, double (pulse counting and analogous) and scanning mode, survey scan and peak jumping. The setting for the survey scan was: the number of runs, 10; dwell time, 0.6 ms; channels per mass, 10; acquisition duration, 13.2 s. The setting for the peak jumping was: sweeps, 25; dwell time, 10 ms; channels per mass, 1; acquisition duration, 34 s.

The element contents in aqueous solutions were determined by the quantitative method using calibration solutions (High Purity Standards, USA) with 5, 10, and 100 μkg/L of each element. Indium was used as an internal standard in all measurements.

The content of Ag, Ba, Cd, Cr,Cu, Dy, Er, Eu, Gd, Hg, Ir, Li, Mo, Nd, Ni, Pb, Pd, Pt, Sb, Se, Sm, Sn, Sr, Te, Ti, Tl, Yb, and Zn in the sample was calculated as a mean value measured by their isotopes. The detection limit (DL) was calculated as:

$$\mathrm{DL} = C_i + 3 \times \mathrm{SD}$$

where C_i is a mean value of the isotope content for measurements in control samples, and SD is a standard deviation of C_i determination in control samples.

For elements with several isotopes, the DL value was used for more abundant isotopes. The results of repeatability (determined as±RSD, a relative standard deviation) did not exceed 10% and 20% for elements with $C_i > 5 \times \mathrm{DL}$ and $C_i < 5 \times \mathrm{DL}$, respectively.

Nine sub-samples of the National Institute Standards and Technologies (NIST, USA) standard reference material SRM NIST 1486 Bone Meal, two sub-samples of certified reference materials Tea Leaves INCT-TL-1, and Mixed Polish Herbs INCT-MPH-2 were analyzed simultaneously with rib samples to estimate the precision and accuracy of results. The samples of standard and certified reference materials were treated in the same way as the rib samples.

In addition, to check the accuracy of measurements the contents of Al, B, Ba, Cu, Li, Mn, Sr, V, and Zn were determined by two independent methods, ICP-AES and ICP-MS and the results were compared. This intermethod examination of each sample increased the reliability of the results.

All rib bone samples were prepared in duplicate and mean values of trace-element contents were used in the final calculation. Using standard programs, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, and percentiles with 0.025 and 0.975 levels were calculated for trace-element contents. The reliability of difference in the results between the female and male cohorts as well as between two age groups was evaluated by Student's t test.

Results

Table 1 depicts our data for Al, As, B, Ba, Br, Cd, Ce, Co, Cr, Cs, Cu, Eu, Hf, Hg, La, Lu, Mn, Mo, Nd, Ni, Pb, Rb, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, Ti, Tl, Tm, U, V, W, Yb, and Zn mass fractions in samples of standard and certified reference materials and the certified (or informative) values of these materials.

Tables 2, 3, and 4 present certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels) of trace-element contents in the rib bone of both females and males, taken separately and together.

The comparison of published data [21–41] with our results for chemical-element contents in the rib bone of women and men is given in Table 5. Because a number of values for chemical element mass fractions were not expressed on a dry-weight basis in the above works, we calculated these values using published data for water and ash contents in the rib bone [42, 43].

We used the entire dataset for both females and males taken separately, seeking to detect the presence of gender-related differences (see Table 6).

To estimate the effect of age on the investigated parameters, we examined two age groups: one comprised a younger group with ages ranging from 15 to 35 years and the other comprised older people with ages ranging from 36 to 55 years. The results for females and males, taken together, are shown in Table 7.

Discussion

Of 38 (Al, As, B, Ba, Br, Cd, Ce, Co, Cr, Cs, Cu, Eu, Hf, Hg, La, Lu, Mn, Mo, Nd, Ni, Pb, Rb, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, Ti, Tl, Tm, U, V, W, Yb, and Zn) trace elements with certified (or informative) values for the used standard and certified reference materials (Table 1), we determined contents of 31 (Al, As, B, Ba, Cd, Ce, Cr, Cs, Cu, Eu, La, Lu, Mn, Mo, Nd, Ni, Pb, Rb, Sb, Sm, Sr, Tb, Th, Ti, Tl, Tm, U, V, W, Yb, and Zn). Mean values for these elements were in the range of 95% confidence interval. The contents of other trace element in the standard and certified reference materials were under detection limit of ICP-MS. A good agreement with the certified data for the reference materials confirms an acceptable accuracy of the results obtained in the study of trace elements of the human rib presented in Tables 2, 3, and 4.

The mean values and all selected statistical parameters were calculated for 23 (Ba, Bi, Cd, Ce, Cu, Dy, Er, Gd, La, Li, Mn, Mo, Nd, Pb, Pr, Rb, Sm, Sr, Tb, Tl, U, Yb, and Zn) trace elements in the human rib bone (Tables 2, 3, and 4). The contents of these elements were measured in all or a major portion of samples. The contents of Ag, Al, B, Be, Br, Cr, Cs, Hg, Ho, Lu, Ni, Sb, Te, Th, Ti, Tm, and Y (17 trace elements) were determined only in a few samples of the collection. The upper limit of 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 (milligram per kilogram on dry-weight basis) of As, Au, Co, Eu, Ga, Hf, Ir, Nb, Pd, Pt, Re, Rh, Sc, Se, Sn, Ta, V, W, and Zr (19 trace elements) were lower than the detection limits of ICP-MS in all samples: As <0.01, Au <0.0007, Co <0.3, Eu <0.0007, Ga <0.2, Hf <0.002, Ir <0.00003, Nb <0.01, Pd <0.01, Pt <0.002, Re <0.0005, Rh <0.01, Sc <0.1, Se <0.2, Sn <0.2, Ta <0.001, V <0.03, W <0.1, and Zr <0.03, respectively.

The obtained means for Al, Ba, Br, Cu, Hg, Li, Ni, Pb, Rb, Sr, Ti, U, V, and Zn, as shown in Table 5, are more or less in agreement with the median of mean values for these

Table 1 ICP-MS data of chemical-element contents (M±SD) in Standard or Certified Reference Materials (milligram per kilogram on dry-weight basis)

Element	Bone meal SRM NIST 1486		Tea leaves (INCT-TL-1)		Mixed Polish herbs INCT-MPH-2	
	Certificate	This work result	Certificate	This work result	Certificate	This work result
Al	<1[a]	≤4	2,290±280	2,842±187	670±111	563±189
As	0.006[a]	<0.01 DL	0.11±0.02	0.089±0.028	0.191±0.023	0.189±0.001
B	–	1.81±0.64	26[a]	26.0±0.7	–	37.6±2.2
Ba	–	270±7	43.2±3.9	43.2±0.2	32.5±2.5	32.5±0.1
Br	–	<7.0 DL	–	<7.0 DL	7.71±0.61	<7.0 DL
Cd	0.003[a]	<0.007	0.030±0.004	0.026±0.001	0.199±0.015	0.232±0.003
Ce	–	0.0272±0.0056	0.790±0.076	0.791±0.027	1.12±0.10	1.26±0.31
Co	–	<0.3 DL	0.39±0.04	<0.3 DL	0.210±0.025	<0.3 DL
Cr	–	≤5	1.9±0.2	2.16±0.29	1.69±0.13	<1.0 DL
Cs	–	<0.003 DL	3.60±0.37	4.10±0.08	0.076±0.007	0.068±0.003
Cu	0.8[a]	1.34±0.28	20.4±1,5	18.0±0.2	7.77±0.53	8.98±0.10
Eu	–	0.0402±0.0027	0.050±0.009	0.046±0.002	0.0157±0.0018	0.0172±0.0028
Hf	–	<0.002 DL	–	<0.002 DL	0.236±0.020	<0.002 DL
Hg	–	<0.03 DL	0.005[a]	<0.03 DL	0.0176±0.0016	<0.03 DL
La	–	0.0211±0.0058	1.00±0.07	0.974±0.012	0.571±0.046	0.616±0.147
Lu	–	0.0007±0.0001	0.017±0.002	0.018±0.001	0.0090±0.0015	0.0037±0.0002
Mn	1[a]	1.02±0.24	1,570±110	1,516±81	191±12	198±3
Mo	–	0.28±0.06	–	0.081±0.020	0.52[a]	0.52±0.01
Nd	–	0.0159±0.0028	0.81[a]	0.858±0.008	0.457±0.091	0.551±0.138
Ni	–	≤3.8	6.1±0.5	6.33±0.43	1.57±0.16	1.53±0.08
Pb	–	1.46±0.14	1.8±0,2	1.68±0.21	2.16±0.23	2.41±0.05
Rb	–	0.266±0.015	81.5±6.5	82.9±2.2	10.7±0.07	10.5±0.3
Sb	–	≤0.011	0.05	0.042±0.005	0.0655±0.0091	0.068±0.002
Sc	–	<0.1 DL	0.27±0.04	<0.1 DL	0.123±0.009	<0.1 DL

Table 1 (continued)

Element	Bone meal SRM NIST 1486		Tea leaves (INCT-TL-1)		Mixed Polish herbs INCT-MPH-2	
	Certificate	This work result	Certificate	This work result	Certificate	This work result
Se	0.13[a]	<0.2 DL	0.076[a]	<0.2 DL	–	<0.2 DL
Sm	–	0.0059±0.0016	0.180±0.022	0.187±0.001	0.0944±0.0082	0.103±0.027
Sr	264±7	251±7	20.8±1.7	20.6±1.7	37.6±2.7	39.7±0.8
Ta	–	<0.001 DL	–	<0.001 DL	0.0186±0.0023	<0.001 DL
Tb	–	0.0013±0.0001	0.027±0.002	0.030±0.002	0.0135±0.0011	0.0115±0.0015
Th	–	0.0040±0.0009	0.034±0.005	0.034±0.001	0.154±0.013	0.158±0.005
Ti	–	≤2.8	30[a]	40±1	34[a]	25.9±1.2
Tl	–	0.0006±0.0001	0.063±0.005	0.060±0.001	0.029[a]	0.030±0.001
Tm	–	0.00033±0.00003	0.017[a]	0.016±0.001	–	0.0041±0.0001
U	–	0.0204±0.0018	–	0.013±0.001	0.049[a]	0.050±0.008
V	–	0.070±0.022	2.0±0.4	2.03±0.07	0.952±0.163	0.928±0.027
W	–	≤0.1	–	<0.1	0.035[a]	<0.1
Yb	–	0.0019±0.0006	0.120±0.013	0.113±0.005	0.0527±0.0066	0.0251±0.0002
Zn	147±16	143±8	34.7±2.7	32.8±3.0	33.5±2.1	38.6±2.4

DL detection limit

[a] Non-certified values

Table 2 Some statistical parameters of trace-element contents (milligram per kilogram on dry-weight basis) in the rib bone of healthy females aged 15–55 years (n=38)

Element	*M*	SD	SEM	Min	Max	Median	P0.025	P0.975
Ag	≤0.011	–	–	<0.007 DL	0.032	–	–	–
Al	≤9.4	–	–	<2.4 DL	11.8	–	–	–
B	≤0.68	–	–	<0.50 DL	2.51	–	–	–
Ba	2.86	1.81	0.29	1.13	7.70	2.34	1.14	7.66
Be	≤0.0021	–	–	<0.001 DL	0.011	–	–	–
Bi	0.0175	0.0182	0.0030	0.001 DL	0.0948	0.0149	0.0017	0.0727
Br	≤3.9	–	–	<3 DL	7.4	–	–	–
Cd	0.0440	0.0414	0.0068	0.005	0.2207	0.0316	0.0050	0.1210
Ce	0.0243	0.0196	0.0032	0.004	0.1092	0.0186	0.0049	0.0607
Cr	≤0.25	–	–	<0.2 DL	1.6	–	–	–
Cs	≤0.011	–	–	<0.003 DL	0.110	–	–	–
Cu	1.00	0.59	0.10	0.48	3.65	0.84	0.59	2.13
Dy	≤0.0012	–	–	<0.0002 DL	0.0027	–	–	–
Er	≤0.00065	–	–	<0.0001 DL	0.0021	–	–	–
Gd	0.00135	0.00106	0.00017	0.0001	0.00545	0.0010	0.0001	0.0048
Hg	<0.01	–	–	<0.01 DL	–	–	–	–
Ho	≤0.00023	–	–	<0.00004 DL	0.00064	–	–	–
La	0.0162	0.0142	0.0023	0.002	0.0828	0.0123	0.0024	0.0417
Li	0.0360	0.0145	0.0024	0.0163	0.068	0.0312	0.0168	0.0667
Lu	≤0.00022	–	–	<0.00006 DL	0.00036	–	–	–
Mn	≤0.430	–	–	<0.1 DL	2.743	–	–	–
Mo	0.0633	0.0542	0.0092	0.017	0.244	0.0493	0.0188	0.2333
Nd	0.0093	0.0081	0.0013	0.0009	0.0455	0.0066	0.0015	0.0241
Ni	≤1.01	–	–	<0.3 DL	5.96	–	–	–
Pb	2.10	1.19	0.19	0.67	5.70	1.87	0.85	5.28
Pr	0.00263	0.00246	0.00041	0.00042	0.01394	0.0022	0.0005	0.0072
Rb	1.39	0.53	0.09	0.70	2.68	1.22	0.71	2.65
Sb	≤0.0068	–	–	<0.004 DL	0.0252	–	–	–
Sm	0.00121	0.00127	0.00021	0.00020	0.00704	0.0008	0.0002	0.0042
Sr	334	209	34	36	1163	294	49	807
Tb	≤0.00029	–	–	<0.0001 DL	0.00078	–	–	–
Te	≤0.0057	–	–	<0.004 DL	0.0240	–	–	–
Th	≤0.0027	–	–	<0.002 DL	0.0056	–	–	–
Ti[a]	≤2.4	–	–	<1.0	8.2	–	–	–
Tl	0.00049	0.00018	0.00003	0.0003	0.00121	0.0005	0.0003	0.0008
Tm	<0.00004	–	–	<0.00004 DL	–	–	–	–
U	0.00111	0.00074	0.00012	0.0005	0.0038	0.0009	0.0005	0.0038
Y	≤0.0038	–	–	<0.002 DL	0.0217	–	–	–
Yb	≤0.00059	–	–	<0.0002 DL	0.0012	–	–	–
Zn	93.2	14.3	2.3	53.6	128	93.5	69.6	114

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *P0.025* percentile with 0.025 level, *P0.975* percentile with 0.975 level

[a] Titanium tools were used for sampling and sample preparation

Table 3 Some statistical parameters of trace-element contents (milligrams per kilogram on dry-weight basis) in the rib bone of healthy males aged 15–55 years (n=42)

Element	*M*	SD	SEM	Min	Max	Median	P0.025	P0.975
Ag	≤0.011	–	–	<0.007 DL	0.040	–	–	–
Al	≤5.1	–	–	<2.5 DL	14.6	–	–	–
B	≤0.63	–	–	<0.5 DL	1.40	–	–	–
Ba	2.24	0.96	0.15	0.57	5.22	2.02	0.88	3.63
Be	≤0.0042	–	–	<0.001 DL	0.0530	–	–	–
Bi	0.0131	0.0111	0.0017	0.0003	0.0587	0.020	0.0003	0.0200
Br	≤3.8	–	–	<3.0 DL	9.4	–	–	–
Cd	0.0442	0.0377	0.0060	0.0107	0.1800	0.0350	0.0124	0.1705
Ce	0.0326	0.0234	0.0036	0.0061	0.1063	0.0222	0.0067	0.0850
Cr	≤0.26	–	–	<0.2 DL	0.85	–	–	–
Cs	≤0.0050	–	–	<0.003 DL	0.0270	–	–	–
Cu	1.10	0.47	0.07	0.40	2.91	1.05	0.40	2.10
Dy	0.00228	0.00135	0.00032	0.00067	0.00554	0.0016	0.0008	0.0048
Er	0.00125	0.00111	0.00026	0.00010	0.0040	0.0001	0.0001	0.0038
Gd	0.00169	0.00114	0.00018	0.00062	0.00580	0.0010	0.0006	0.0047
Hg	≤0.018	–	–	<0.01 DL	0.050	–	–	–
Ho	≤0.00056	–	–	<0.00004 DL	0.00092	–	–	–
La	0.0228	0.0196	0.0031	0.0056	0.1106	0.0170	0.0061	0.0550
Li	0.0376	0.0127	0.0020	0.0177	0.0726	0.0357	0.0224	0.0706
Lu	≤0.00026	–	–	<0.00006 DL	0.00080	–	–	–
Mn	0.273	0.255	0.043	0.050	1.600	0.200	0.104	0.760
Mo	0.0374	0.0255	0.0051	0.0190	0.1338	0.0290	0.0190	0.0931
Nd	0.0119	0.0103	0.0016	0.0022	0.0615	0.0085	0.0037	0.0330
Ni	≤1.07	–	–	<0.3 DL	6.20	–	–	–
Pb	2.36	1.22	0.20	1.00	6.70	2.08	1.06	5.88
Pr	0.00379	0.00397	0.00062	0.00089	0.0210	0.0023	0.0010	0.0161
Rb	1.62	0.56	0.09	0.77	3.00	1.50	0.93	2.82
Sb	≤0.012	–	–	<0.004 DL	0.172	–	–	–
Sm	0.00162	0.00119	0.00019	0.00020	0.00520	0.0014	0.0006	0.0047
Sr	252	137	21	58	701	245	63	576
Tb	0.00043	0.00021	0.00003	0.00010	0.0011	0.0004	0.0001	0.0009
Te	<0.004	–	–	<0.004 DL	–	–	–	–
Th	≤0.0033	–	–	<0.002 DL	0.0065	–	–	–
Ti[a]	<3.1	–	–	<1.0	13.4	–	–	–
Tl	0.00050	0.00029	0.00005	0.0001	0.0018	0.0004	0.0003	0.0009
Tm	≤0.00008	–	–	<0.00004 DL	0.00029	–	–	–
U	0.00142	0.00095	0.00015	0.0005	0.0040	0.0010	0.0005	0.0039
Y	≤0.0055	–	–	<0.002 DL	0.0201			
Yb	0.00087	0.00062	0.00013	0.0001	0.0024	0.0006	0.0002	0.0021
Zn	92.5	13.3	2.1	63.4	123.7	90.8	73.4	112

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *P0.025* percentile with 0.025 level, *P0.975* percentile with 0.975 level

[a] Titanium tools were used for sampling and sample preparation

Table 4 Some statistical parameters of trace-element contents (milligrams per kilogram on dry-weight basis) in the rib bone of both healthy females and males aged 15–55 years, taken together (n=80)

Element	*M*	SD	SEM	Min	Max	Median	P0.025	P0.975
Ag	≤0.011	–	–	<0.007 DL	0.040	–	–	–
Al	≤7.2	–	–	<2.4 DL	14.6	–	–	–
B	≤0.65	–	–	<0.5 DL	2.51	–	–	–
Ba	2.54	1.46	0.16	0.57	7.70	2.34	1.09	7.41
Be	≤0.0032	–	–	<0.001 DL	0.0530	–	–	–
Bi	0.0153	0.0150	0.0017	0.0003	0.0948	0.0155	0.0006	0.0593
Br	≤3.9	–	–	<3.0 DL	9.4	–	–	–
Cd	0.0441	0.0393	0.0045	0.0050	0.2207	0.0317	0.0058	0.1713
Ce	0.0286	0.0219	0.0024	0.0040	0.1092	0.0220	0.0057	0.0861
Cr	≤0.25	–	–	<0.2 DL	1.55	–	–	–
Cs	≤0.0077	–	–	<0.003 DL	0.1100	–	–	–
Cu	1.05	0.53	0.06	0.40	3.65	1.00	0.47	2.16
Dy	0.00204	0.00135	0.00028	0.0002	0.00554	0.0015	0.0002	0.0046
Er	0.00112	0.00107	0.00022	0.0001	0.0040	0.0007	0.0001	0.0037
Gd	0.00153	0.00111	0.00012	0.00010	0.00580	0.0010	0.0001	0.0048
Hg	≤0.018	–	–	<0.01 DL	0.050	–	–	–
Ho	≤0.00053	–	–	<0.00004 DL	0.00090	–	–	–
La	0.0197	0.0174	0.0019	0.0020	0.1106	0.0143	0.0043	0.0564
Li	0.0369	0.0135	0.0015	0.0163	0.0726	0.0343	0.0170	0.0683
Lu	≤0.00024	–	–	<0.00006 DL	0.00080	–	–	–
Mn	0.354	0.353	0.0041	0.050	2.74	0.200	0.110	0.808
Mo	0.052	0.046	0.006	0.017	0.244	0.038	0.019	0.187
Nd	0.0107	0.0094	0.0011	0.0009	0.0615	0.0082	0.0022	0.0336
Ni	≤1.05	–	–	<0.3 DL	6.20	–	–	–
Pb	2.24	1.21	0.14	0.67	6.70	1.91	0.89	5.71
Pr	0.00324	0.00337	0.00038	0.00042	0.02100	0.0023	0.0007	0.0141
Rb	1.51	0.55	0.06	0.70	3.00	1.44	0.76	2.72
Sb	≤0.0096	–	–	<0.004 DL	0.1720	–	–	–
Sm	0.0014	0.0012	0.0001	0.0002	0.0070	0.0008	0.0002	0.0047
Sr	291	179	20	36	1163	271	58	705
Tb	0.00041	0.00027	0.00005	0.0001	0.00111	0.0004	0.0001	0.0009
Te	≤0.0057	–	–	<0.004 DL	0.0240	–	–	–
Th	≤0.0030	–	–	<0.002 DL	0.0065	–	–	–
Ti[a]	≤2.8	–	–	<1.0 DL	13.4	–	–	–
Tl	0.00050	0.00024	0.00003	0.0001	0.0018	0.0004	0.0003	0.0009
Tm	≤0.00006	–	–	<0.00004 DL	0.00029	–	–	–
U	0.00127	0.00087	0.00010	0.0005	0.0040	0.0010	0.0005	0.0038
Y	≤0.0047	–	–	<0.0020 DL	0.0217	–	–	–
Yb	0.00072	0.00047	0.00007	0.0001	0.0024	0.0006	0.0002	0.0019
Zn	92.8	13.7	1.5	53.6	128	92.4	70.5	113

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *P0.025* percentile with 0.025 level, *P0.975* percentile with 0.975 level

[a] Titanium tools were used for sampling and sample preparation

Table 5 Median, minimum and maximum value of means of trace-element contents (milligrams per kilogram on dry-weight basis) in human rib bone according to data from the literature in comparison with our results

Element	Published data [21–41]			This work result
	Median (*n*)	Minimum [reference]	Maximum [reference]	*M*±SEM
Ag	0.16 (3)	<0.03 [21]	0.52 [22]	≤0.011
Al	28 (4)	3.8 [23]	41.0 [24]	≤7.20
As	0.06 (4)	0.02 [25]	0.5 [26]	<0.01 DL
Au	≤0.014 (3)	<0.01 [21]	0.1 [26]	<0.001 DL
B	3.8 (1)	2.9 [22]	4.8 [22]	≤0.65
Ba	9.1 (10)	0.94 [27]	36 [28]	2.54±0.16
Bi	≤0.1 (2)	<0.009 [22]	<0.2 [26]	0.015±0.002
Br	4.8 (5)	0.83 [29]	≤24 [30]	≤3.9
Cd	1.1 (10)	0.09 [24]	2.7 [28]	0.044±0.005
Ce	1.4 (2)	<0.03 [21]	2.7 [31]	0.029±0.002
Co	0.13 (7)	0.0019 [21]	21.3 [25]	<0.3 DL
Cr	3.3 (8)	0.098 [32]	15.9 [25]	≤0.25
Cs	0.038 (4)	<0.004 [26]	0.047 [22]	≤0.0077
Cu	6.3 (18)	0.19 [26]	12.9 [25]	1.05±0.06
Dy	≤0.2 (2)	<0.01 [26]	<0.4 [22]	0.0020±0.0003
Er	≤0.17 (2)	<0.01 [26]	<0.33 [22]	0.0011±0.0002
Eu	≤0.038 (4)	<0.001 [21]	<0.19 [22]	<0.0007 DL
Ga	<0.03 (1)	<0.03 [26]	<0.03 [26]	<0.2 DL
Gd	≤0.25 (3)	<0.008 [26]	<0.47 [22]	0.0015±0.0001
Hf	≤0.11 (2)	<0.02 [21]	<0.2 [26]	<0.002 DL
Hg	≤0.033 (3)	<0.008 [21]	3.3 [31]	≤0.018
Ho	≤0.047 (2)	<0.004 [26]	<0.09 [22]	≤0.00053
La	≤0.3 (4)	<0.05 [21]	0.86 [31]	0.020±0.002
Li	<0.05 (1)	<0.05 [26]	<0.05 [26]	0.0369±0.0015
Lu	≤0.004 (3)	<0.003 [21]	<0.09 [22]	≤0.00024
Mn	3.6 (15)	<0.13 [26]	9.7 [33]	0.354±0.041
Mo	15 (4)	<0.06 [32]	51.6 [25]	0.107±0.032
Nb	≤0.07 (2)	<0.03 [22]	<0.1 [26]	<0.01 DL
Nd	≤0.1 (3)	<0.02 [26]	<0.33 [22]	0.011±0.001
Ni	2.6 (8)	0.37 [32]	55.1 [25]	≤1.05
Pb	11.9 (35)	0.56 [32]	93 [34]	2.24±0.14
Pr	≤0.05 (2)	<0.01 [26]	<0.09 [22]	0.0032±0.0004
Pt	<0.06 (1)	<0.06 [26]	<0.06 [26]	<0.002 DL
Rb	1.82 (5)	<0.08 [26]	3.59 [35]	1.51±0.06
Re	<0.004 (1)	<0.004 [26]	<0.004 [26]	<0.0005 DL
Sb	≤0.61 (3)	<0.01 [21]	<0.80 [22]	≤0.0096
Sc	≤0.093 (2)	<0.001 [21]	0.186 [31]	<0.1 DL
Se	≤1.0 (2)	<0.03 [21]	<2.0 [26]	<0.2 DL
Sm	≤0.01 (3)	<0.004 [26]	<0.4 [22]	0.0014±0.0001
Sn	1.9 (4)	<0.79 [26]	7.1 [37]	<0.2 DL

Table 5 (continued)

Element	Published data [21–41]			This work result
	Median (*n*)	Minimum [reference]	Maximum [reference]	*M*±SEM
Sr	80.2 (13)	53.4 [36]	400 [31]	291±20
Ta	≤0.0065 (2)	<0.005 [21]	<0.008 [26]	<0.001 DL
Tb	≤0.03 (3)	<0.004 [26]	<0.09 [22]	0.00041±0.00005
Te	<0.3 (1)	<0.3 [26]	<0.3 [26]	≤0.0057
Th	≤0.05 (3)	<0.019 [22]	<0.06 [26]	≤0.0030
Ti	≤4 (2)	<3 [26]	<5 [24]	≤2.8
Tl	≤0.14 (2)	<0.008 [26]	<0.28 [22]	0.00050±0.00003
Tm	≤0.047 (2)	<0.004 [26]	<0.09 [22]	≤0.00006
U	0.0044 (7)	0.003 [38]	<0.0094 [22]	0.00127±0.00010
V	≤0.16 (5)	0.0033 [39]	0.32 [32]	<0.03DL
W	<0.08 (1)	<0.08 [26]	<0.08 [26]	<0.1 DL
Y	0.033 (3)	<0.03 [26]	≤40 [30]	≤0.0047
Yb	≤0.03 (3)	<0.004 [26]	<0.33 [22]	0.00072±0.00007
Zn	102 (22)	26 [40]	220±40 [41]	92.8±1.5
Zr	≤0.2 (3)	<0.05 [22]	<0.3 [26]	<0.03 DL

n number of all references, *M* arithmetic mean, *SEM* standard error of mean

elements in the human rib bone previously published in [21–41]. The means or upper limit of means for Ag, B, Bi, Cd, Ce, Cr, Cs, Dy, Er, Eu, Gd, Ho, La, Lu, Mn, Mo, Nd, Pr, Re, Sb, Sm, Sn, Tb, Te, Th, Tl, Tm, Y, and Yb are one to two orders of magnitude lower, than previously reported results. High values of Cr, Mn, and Mo among these elements can be explained by contamination during either sampling or sample preparation when instruments made of stainless steel were used. The detection limits of ICP-MS show that the contents of As, Eu, Nb, Sn, Ta, W, and Zr in intact rib bones of healthy men are nearly equal, and those of Au, Hf, Pt, and Re are at least one order of magnitude lower, than the previously reported lower limit of means. The measured mean value for Ce, Cr, Cs, Hg, La, Rb, Sb, Sr, and Zn contents in the human rib are in good agreement with our previously published result found by instrumental neutron activation analysis [21, 42].

It was pointed out by Anke et al. [33], Schroeder et al. [27], Yoshinaga et al. [24, 26] that in women rib bone, the contents of Sr are higher than in men's. Our results are indicative of the same tendency (Table 6). 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 in the Russian Central European region consume more plant foods, which is the main supplier of Sr in the human body.

With the exception of Mo and Sr we did not find any differences in the content of trace elements in female and male rib. Therefore, the search for the age-related differences was continued in the combined cohort of females and males. Statistically significant, an age-related increase in Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb, and Yb was observed when studied cohorts of females and males were analyzed together (see Table 7). For the first time, it was shown that there is an age-dependent increase in the content of Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb, and Yb in the rib of a healthy person living in an environmentally prosperous region.

Table 6 Effect of gender on mean value (M±SEM) of trace-element contents in the intact human rib bone (milligrams per kilogram on dry-weight basis)

Element	Effect of gender		
	Females n=38	Males n=42	p t test
Ag	≤0.011	≤0.011	N.S.
Al	≤9.4	≤5.1	N.S.
B	≤0.68	≤0.63	N.S.
Ba	2.86±0.29	2.24±0.15	N.S.
Be	≤0.0021	≤0.0042	N.S.
Bi	0.018±0.003	0.013±0.002	N.S.
Br	≤3.9	≤3.8	N.S.
Cd	0.044±0.007	0.044±0.006	N.S.
Ce	0.024±0.003	0.033±0.004	N.S.
Cr	≤0.25	≤0.26	N.S.
Cs	≤0.0106	≤0.0050	N.S.
Cu	1.00±0.10	1.10±0.07	N.S.
Dy	≤0.0012	0.0023±0.0003	N.S.
Er	≤0.0065	0.0013±0.0003	N.S.
Gd	0.0014±0.0002	0.0017±0.0002	N.S.
Hg	≤0.010	≤0.018	N.S.
Ho	≤0.00023	≤0.00056	N.S.
La	0.016±0.002	0.023±0.003	N.S.
Li	0.036±0.002	0.038±0.002	N.S.
Lu	≤0.00022	≤0.00026	N.S.
Mn	≤0.430	0.270±0.005	N.S.
Mo	0.063±0.009	0.037±0.005	≤0.05
Nd	0.0093±0.0013	0.0120±0.0016	N.S.
Ni	≤1.01	≤1.07	N.S.
Pb	2.10±0.19	2.36±0.20	N.S.
Pr	0.0026±0.0004	0.0038±0.0006	N.S.
Rb	1.39±0.09	1.62±0.09	N.S.
Sb	≤0.0068	≤0.012	N.S.
Sm	0.0012±0.0002	0.0016±0.0002	N.S.
Sr	334±34	252±21	≤0.05
Tb	≤0.00029	0.00043±0.00005	N.S.
Te	≤0.0057	<0.004	N.S.
Th	≤0.0027	≤0.0033	N.S.
Ti[a]	≤2.4	≤3.1	N.S.
Tl	0.00049±0.00003	0.00050±0.00005	N.S.
Tm	≤0.00004	≤0.00008	N.S.
U	0.00111±0.00012	0.00142±0.00015	N.S.
Y	≤0.0038	≤0.0055	N.S.
Yb	≤0.00059	0.00087±0.00013	N.S.
Zn	93.2±2.3	92.5±2.1	N.S.

M arithmetic mean, *SEM* standard error of mean, *N.S.* non significant

[a] Titanium tools were used for sampling and sample preparation

Table 7 Effect of age on mean value (*M*±SEM) of trace-element contents in the intact human rib bone (milligrams per kilogram on dry-weight basis)

Element	Effect of age		
	15–35 year n=36	36–55 year n=44	p t test
Ag	≤0.013	≤0.010	N.S.
Al	≤6.6	≤7.7	N.S.
B	≤0.58	≤0.70	N.S.
Ba	2.45±0.23	2.61±0.23	N.S.
Be	≤0.0027	≤0.0036	N.S.
Bi	0.017±0.003	0.014±0.002	N.S.
Br	≤3.6	≤4.1	N.S.
Cd	0.037±0.006	0.049±0.006	N.S.
Ce	0.018±0.002	0.037±0.004	≤0.001
Cr	≤0.23	≤0.27	N.S.
Cs	≤0.0094	≤0.0064	N.S.
Cu	1.09±0.09	1.02±0.08	N.S.
Dy	0.0010±0.0003	0.0025±0.0003	≤0.001
Er	0.00053±0.00026	0.00138±0.00028	≤0.05
Gd	0.00090±0.00006	0.00203±0.00019	≤0.001
Hg	≤0.019	≤0.018	N.S.
Ho	≤0.00051	<0.00054	N.S.
La	0.012±0.001	0.026±0.003	≤0.001
Li	0.034±0.002	0.039±0.002	N.S.
Lu	≤0.00024	≤0.00024	N.S.
Mn	0.36±0.08	0.35±0.04	N.S.
Mo	0.064±0.012	0.044±0.005	N.S.
Nd	0.0064±0.0007	0.0141±0.0016	≤0.001
Ni	≤1.02	≤1.06	N.S.
Pb	2.14±0.25	2.31±0.16	N.S.
Pr	0.0017±0.0002	0.0044±0.0006	≤0.001
Rb	1.49±0.09	1.53±0.09	N.S.
Sb	≤0.012	≤0.008	N.S.
Sm	0.00072±0.00005	0.00198±0.00021	≤0.001
Sr	301±39	284±20	N.S.
Tb	0.00025±0.00006	0.00049±0.00006	≤0.01
Te	<0.004	≤0.0057	N.S.
Th	<0.002	≤0.0031	N.S.
Ti[a]	≤2.2	≤3.2	N.S.
Tl	0.00047±0.00003	0.00052±0.00004	N.S.
Tm	≤0.00004	≤0.00010	N.S.
U	0.0011±0.0001	0.0014±0.0001	N.S.
Y	<0.0035	≤0.0056	N.S.
Yb	0.00056±0.00006	0.00081±0.00009	≤0.05
Zn	91.6±1.9	93.8±2.3	N.S.

M arithmetic mean, *SEM* standard error of mean, *N.S.* non significant

[a] Titanium tools were used for sampling and sample preparation

Table 8 Correlations (*r*) of trace-element contents in the human rib bone

	Bi	Cd	Ce	Cu	Dy	Er	Gd	La	Li	Mn	Mo	Nd	Pb	Pr	Rb	Sm	Sr	Tb	Tl	U	Yb	Zn
Ba	**0.31**	0.18	−0.01	−0.19	0.03	0.06	−0.11	0.01	−0.05	0.09	−0.08	−0.06	−0.04	−0.12	−0.14	−0.16	0.15	0.04	0.02	−0.13	−0.15	0.19
Bi	XX	0.05	−0.11	−0.04	0.29	−0.07	−0.13	−0.03	−0.02	−0.10	0.01	−0.11	0.04	−0.11	−0.07	−0.13	0.13	0.11	−0.05	−0.09	0.02	0.06
Cd		XX	0.01	0.10	−0.06	−0.08	−0.05	0.02	0.01	0.09	−0.06	−0.06	−0.08	−0.07	−0.08	−0.09	0.15	0.04	−0.05	−0.01	−0.03	0.10
Ce			XX	−0.13	0.24	**0.32**	**0.74**	**0.89**	0.15	0.09	−0.09	**0.91**	−0.10	**0.63**	−0.14	**0.83**	0.05	**0.46**	0.15	**0.30**	0.13	0.01
Cu				XX	−0.02	0.05	−0.03	−0.13	−0.14	−0.11	**0.58**	−0.12	**0.25**	−0.13	0.18	−0.03	−0.07	−0.03	−0.01	0.11	0.20	−0.12
Dy					XX	**0.50**	**0.32**	**0.32**	0.02	0.08	0.04	0.23	0.20	0.19	−0.04	**0.26**	0.10	**0.60**	0.14	0.13	**0.70**	0.06
Er						XX	**0.38**	**0.26**	0.08	0.05	−0.04	**0.27**	0.02	0.22	−0.17	**0.31**	0.03	**0.54**	0.11	0.14	**0.42**	0.09
Gd							XX	**0.63**	0.19	0.08	−0.06	**0.71**	−0.01	**0.50**	−0.02	**0.90**	0.07	**0.63**	**0.45**	**0.51**	**0.27**	0.04
La								XX	0.18	0.13	−0.11	**0.92**	−0.08	**0.61**	−0.16	**0.75**	0.09	**0.55**	0.24	0.20	0.18	0.01
Li									XX	−0.04	0.01	0.09	−0.04	−0.04	−0.10	0.16	0.13	0.23	0.24	**0.34**	0.03	0.22
Mn										XX	−0.20	0.16	−0.20	0.19	0.01	0.08	0.01	0.17	0.22	0.01	0.17	−0.09
Mo											XX	−0.08	**0.55**	−0.06	−0.07	−0.04	−0.05	−0.04	−0.04	−0.02	0.09	0.09
Nd												XX	−0.06	**0.69**	−0.17	**0.84**	0.05	**0.62**	**0.29**	0.20	0.15	0.01
Pb													XX	−0.07	−0.10	−0.04	−0.06	0.06	−0.01	−0.04	0.10	0.02
Pr														XX	−0.04	**0.60**	−0.07	**0.36**	0.18	0.09	0.16	−0.08
Rb															XX	−0.03	−0.10	−0.19	0.08	0.07	−0.09	−0.10
Sm																XX	0.02	**0.56**	**0.37**	**0.39**	0.21	0.01
Sr																	XX	0.17	0.04	0.14	0.05	**0.23**
Tb																		XX	**0.27**	**0.35**	**0.51**	0.22
Tl																			XX	**0.30**	0.22	−0.09
U																				XX	0.12	0.18
Yb																					XX	−0.03
Zn																						XX

bold statistically significant values

The standard deviation obtained for all trace elements are respectively large (Tables 2, 3, 4, 6, and 7). This is due to the very wide individual variation of trace element mass fractions in the human rib bone.

The intercorrelations of the chemical-element contents in the bone tissue are a very interesting topic of the bone physiology and pathology. Intercorrelation calculations including all chemical elements identified by us, including the previously published data [20], are in progress (Table 8).

Conclusions

The inductively coupled plasma mass spectrometry allows the determination of contents or an upper limit of contents of 59 trace elements in human rib bone samples of healthy adults. Mean values (M±SEM) for the mass fraction of Ba, Bi, Cd, Ce, Cu, Dy, Er, Gd, La, Li, Mn, Mo, Nd, Pb, Pr, Rb, Sm, Sr, Tb, Tl, U, Yb, and Zn (milligrams per kilogram of dry bone) were: 2.5±0.2, 0.015±0.002, 0.044±0.005, 0.029±0.002, 1.05±0.06, 0.0020±0.0003, 0.0011±0.0002, 0.0015±0.0001, 0.020±0,002, 0.040±0.002, 0.354±0.004, 0.052±0.006, 0.011±0.001, 2.24±0.14, 0.0032±0.0004, 1.51±0.06, 0.0014±0.0001, 291±20, 0.00041±0.00005, 0.00050±0.00003, 0.0013±0.0001, 0.00072±0.00007, and 92.8±1.5, respectively. The upper limit of mean contents of Ag, Al, B, Be, Br, Cr, Cs, Hg, Ho, Lu, Ni, Sb, Te, Th, Ti, Tm, and Y were: ≤0.011, ≤7.2, ≤0.65, ≤0.0032, ≤3.9, ≤0.25, ≤0.0077, ≤0.018, ≤0.00053, ≤0.00024, ≤1.05, ≤0.0096, ≤0.0057, ≤0.0030, ≤2.8, ≤0.00006, and≤0.0047, respectively. In all rib bone samples the contents of As, Au, Co, Eu, Ga, Hf, Ir, Nb, Pd, Pt, Re, Rh, Sc, Se, Sn, Ta, V, W, and Zr were under detection limits (DL): 0.01, 0.001, 0.3, 0.0007, 0.2, 0.002, 0.0001, 0.01, 0.01, 0.002, 0.0005, 0.02, 0.1, 0.2, 0.2, 0.001, 0.03, 0.1, and 0.03, respectively. Statistically, significant tendency for the in Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb, and Yb content to increase with age was found in the human rib bone, regardless of gender. It was shown that higher Sr mass fractions were typical of female ribs as compared to those in male ribs.

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 59 trace-element contents in the intact rib bone may serve as indicative normal values for residents of the Central European region of Russia.

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