RELATIONSHIP BETWEEN Ca, Cl, K, Mg, Mn, Na, P, AND Sr CONTENTS IN THE HUMAN RIB BONE INVESTIGATED BY NEUTRON ACTIVATION ANALYSIS

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Introduction

The bioaccumulation of chemical elements in human bone is rather a complex process. Factors that influence bioaccumulation include age, gender, genetic inheritance, dietary habits, environmental quality, and so on. Many chemical elements in human organism act antagonistically and/or synergistically. Some elements in the bone can be substituted by other elements and, as a result, change biochemical reactions in humans. Variations in relative content of chemical elements in the bone lead to modulation/dysfunction of bone metabolism.

To use chemical element composition as estimation of bone health in clinical, geographical, environmental and occupational medicine, paleoanthropology, and other directions, it is necessary to know normal levels and age- and gender-related changes of chemical element ratios.

This work had three aims. The first one was to determine the Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the normal human rib bone by instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and to calculate some statistical parameters of Cl/Ca, K/Ca, Mg/Ca, Mn/Ca, Na/Ca, P/Ca, Sr/Ca, Ca/P, Cl/P, K/P, Mg/P, Mn/P, Na/P, Sr/P, Ca/Mg, Cl/Mg, Mn/Mg, Na/Mg, P/Mg, Sr/Mg, Ca/Cl, K/Cl, Mg/Cl, Mn/Cl, Na/Cl, P/Cl, Sr/Cl, Ca/K, Cl/K, Mg/K, Mn/K, Na/K, P/K, Sr/K, Ca/Na, Cl/Na, K/Na, Mg/Na, Mn/Na, P/Na, and Sr/Na mass fraction ratios. The second aim was to evaluate the effect of age and gender on mean values of ratios of chemical element mass fractions in intact human rib bone. The third aim was to estimate the inter correlations between Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the normal human rib bone.

All studies were approved by the Medical Radiological Research Center, Obninsk, Ethical Committees.

Experimental

Rib bone samples were obtained at postmortems from intact cadavers (38 female and 46 male, 15–55 years old) within 24 h of death. The bone samples were immediately frozen at -18 ºC until use. All subjects died suddenly due to automobile accident, falls, shootings, stabbing, hanging, acute alcohol poisoning, or hypothermia. 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 cross sections of the rib weighing about 50–100 mg

and containing cortical and trabecular parts in natural ratio. The rib samples for INAA-SLR were sealed separately in thin polyethylene films washed with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules.

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol–formaldehyde resins were used [1]. Corrected certified values of BSS element contents were reported by us before [2]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten CRM IAEA H-5 (Animal Bone) and SRM NIST 1486 (Bone Meal) sub-samples weighing about 50–100 mg were analyzed in the same conditions as bone samples to estimate the precision and accuracy of results.

The contents of Ca, Cl, K, Mg, Mn, Na, P, and Sr were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor. The neutron flux in the channel was 1.7×10^{13} n cm^{-2} s^{-1}. Ampoules with bone samples, BSS, intralaboratory-made standards, and certified reference materials were put into polyethylene rabbits and then irradiated separately for 30 s. Copper foils were used to assess neutron flux. The measurement of each sample was made twice, 1 and 120 min after irradiation. The duration of the first and second measurements was 10 and 20 min, respectively. A coaxial 98 cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100), including a PC-coupled multichannel analyzer, were used for measurements. The spectrometric unit provided 2.9 keV resolution at the ^{60}Co 1332 keV line. The information of used nuclear reactions, radionuclides, gamma-energies, and other details of the analysis including the quality control of results were reported by us before [3].

A dedicated computer program of NAA mode optimization was used [4]. 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 different ratios of chemical element mass fractions. The reliability of difference in the results between two age groups and between females and males was evaluated by Student's t-test. A correlation analysis was used to identify relationships between elements.

Results and discussion

Tables 1-3 represent 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 42 different ratios of Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the normal rib bone of males and females, and of both females and males, taken together.

The obtained values for Ca/P ratio, as shown in Tables 1-3, agree well with median of means cited by other researchers for the human rib bone [5-7]. No published data referring to ratios of other chemical element mass fractions in human rib bone was found.

To estimate the effect of age on the chemical element ratios in rib bone 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). No changes with age in Ca/P ratio in human rib bone were demonstrated in previously published studies [5-7]. No published data referring to age-related changes of ratios of other chemical element mass fractions in human rib bone were found.

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

Table 1. Some statistical parameters of 42 different ratios of Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the normal male rib bone

Ratio	M	SD	SEM	Min	Max	Med	P0.025	P0.975
(Cl/Ca)×10^3	6.32	2.49	0.38	3.19	13.4	5.68	3.81	12.8
(K/Ca) ×10^3	5.84	2.71	0.41	1.17	13.1	5.85	1.64	10.8
(Mg/Ca) ×10^2	1.21	0.27	0.04	0.560	2.15	1.18	0.773	1.63
(Mn/Ca) ×10^6	1.56	0.91	0.14	0.303	4.70	1.30	0.418	3.53
(Na/Ca) ×10^2	2.61	0.53	0.08	1.28	4.56	2.55	1.92	3.75
P/Ca	0.433	0.061	0.009	0.319	0.634	0.420	0.331	0.528
(Sr/Ca) ×10^3	1.45	0.66	0.10	0.284	2.92	1.38	0.352	2.62
Ca/P	2.35	0.32	0.05	1.58	3.13	2.38	1.89	3.02
(Cl/P) ×10^2	1.47	0.55	0.08	0.635	3.11	1.34	0.733	2.59
(K/P) ×10^2	1.38	0.66	0.10	0.272	3.17	1.39	0.392	2.67
(Mg/P) ×10^2	2.82	0.59	0.09	1.12	4.19	2.85	1.86	3.86
(Mn/P) ×10^6	3.59	2,23	0.35	0.724	11.9	3.19	0.932	9.23
(Na/P) ×10^2	6.18	1.69	0.26	2.73	13.6	5.93	3.10	8.19
(Sr/P) ×10^3	3.38	1.53	0.23	0.685	7.01	3.33	0.829	6.35
Ca/Mg	86.9	22.1	3.3	46.6	178	84.9	61.4	129
Cl/Mg	0.537	0.207	0.031	0.237	1.16	0.537	0.252	0.993
K/Mg	0.493	0.236	0.036	0.120	1.12	0.489	0.143	0.951
(Mn/Mg) ×10^4	1.35	0.88	0.14	0.224	4.46	1.16	0.258	3.28
Na/Mg	2.24	0.62	0.09	1.18	4.27	2.18	1.28	3.74
P/Mg	37.4	10.7	1.6	23.9	89.2	35.0	25.9	53.8
Sr/Mg	0.125	0.066	0.010	0.030	0.378	0.123	0.031	0.244
Ca/Cl	179	58.3	8.8	74.8	314	176	78.2	263
K/Cl	0.963	0.441	0.067	0.263	1.94	0.850	0.427	1.92
Mg/Cl	2.15	0.84	0.13	0.861	4.22	1.86	1.01	3.97
(Mn/Cl) ×10^4	2.61	1.45	0.23	0.686	6.34	2.37	0.745	6.08
Na/Cl	4.53	1.33	0.20	2.12	7.55	4.51	2.15	7.30
P/Cl	77.2	27.5	4.1	32.1	157	74.6	38.6	137
Sr/Cl	0.248	0.133	0.020	0.042	0.541	0.214	0.067	0.516
Ca/K	228	154	23	76.4	855	171	92.3	610
Cl/K	1.27	0.63	0.095	0.516	3.80	1.18	0.520	2.34
Mg/K	2.65	1.59	0.24	0.892	8.31	2.04	1.05	7.04
(Mn/K) ×10^4	3.11	1.92	0.30	0.541	9.00	2.91	0.676	6.80
Na/K	5.60	3.42	0.52	2.26	21.7	4.51	2.62	14.1
P/K	99.0	68.4	10.3	31.6	367	71.7	37.5	255
Sr/K	0.303	0.237	0.036	0.027	1.19	0.245	0.103	1.09
Ca/Na	39.8	8.3	1.3	21.0	77.9	39.2	26.7	52.1
Cl/Na	0.242	0.080	0.012	0.133	0.471	0.222	0.137	0.465
K/Na	0.220	0.087	0.013	0.046	0.443	0.222	0.071	0.381
Mg/Na	0.478	0.131	0.020	0.234	0.848	0.458	0.267	0.780
(Mn/Na) ×10^4	0.614	0.349	0.055	0.130	1.80	0.548	0.191	1.30
P/Na	17.4	5.06	0.76	7.37	36.7	16.9	12.2	32.5
Sr/Na	0.559	0.270	0.041	0.119	1.41	0.509	0.124	1.06

M - arithmetic mean; SD – standard deviation; SEM – standard error of mean; Min – minimum value; Max – maximum value; Per. 0.025 – percentile with 0.025 level; Per. 0.975 – percentile with 0.975 level

Table 2. Some statistical parameters of 42 different ratios of Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the normal female rib bone

Ratio	M	SD	SEM	Min	Max	Med	P0.025	P0.975
(Cl/Ca)×10^3	4.26	2.68	0.44	0.699	13.6	4.18	0.749	11.0
(K/Ca) ×10^3	3.21	1.96	0.35	0.214	8.67	3.10	0.533	7.20
(Mg/Ca) ×10^2	0.977	0.338	0.056	0.355	1.61	1.01	0.422	1.48
(Mn/Ca) ×10^6	1.62	1.30	0.22	0.276	7.18	1.26	0.318	4.84
(Na/Ca) ×10^2	2.41	0.58	0.096	0.860	3.88	2.38	0.885	3.38
P/Ca	0.444	0.073	0.012	0.264	0.708	0.432	0.358	0.581
(Sr/Ca) ×10^3	1.60	1.03	0.17	0.140	5.88	1.35	0.314	3.27
Ca/P	2.31	0.38	0.062	1.41	3.79	2.31	1.73	2.82
(Cl/P) ×10^2	1.00	0.68	0.11	0.141	3.35	0.883	0.152	2.67
(K/P) ×10^2	0.763	0.483	0.085	0.052	2.11	0.724	0.114	1.64
(Mg/P) ×10^2	2.29	0.97	0.16	0.756	5.56	2.48	0.797	4.13
(Mn/P) ×10^6	3.62	2.60	0.43	0.627	12.7	2.96	0.773	11.2
(Na/P) ×10^2	5.57	1.68	0.28	1.77	11.4	5.64	2.09	8.70
(Sr/P) ×10^3	3.71	2.54	0.42	0.352	13.3	2.88	0.749	9.60
Ca/Mg	119	53	8.7	62.2	282	98.9	67.6	238
Cl/Mg	0.423	0.178	0.029	0.124	0.911	0.401	0.171	0.851
K/Mg	0.302	0.156	0.028	0.028	0.738	0.256	0.099	0.630
(Mn/Mg) ×10^4	2.13	2.61	0.43	0.212	12.8	1.34	0.365	10.4
Na/Mg	2.91	1.59	0.26	0.535	7.75	2.49	1.03	6.43
P/Mg	53.6	27.8	4.5	18.0	132	40.3	24.5	126
Sr/Mg	0.181	0.120	0.020	0.012	0.529	0.159	0.025	0.498
Ca/Cl	357	295	49	73.7	1431	239	91.4	1335
K/Cl	0.733	0.302	0.053	0.051	1.36	0.704	0.270	1.33
Mg/Cl	2.85	1.43	0.23	1.10	8.09	2.50	1.18	5.93
(Mn/Cl) ×10^4	6.16	8.62	1.44	0.772	39.1	3.26	0.998	33.5
Na/Cl	8.82	7.96	1.31	0.634	36.7	6.27	1.97	35.8
P/Cl	162	148	24.3	29.9	710	113	37.6	656
Sr/Cl	0.536	0.634	0.104	0.063	3.78	0.376	0.064	1.84
Ca/K	598	829	147	115	4662	322	140	2288
Cl/K	2.09	3.25	0.57	0.738	19.6	1.42	0.755	6.73
Mg/K	4.98	6.00	1.06	1.35	36.3	3.90	1.60	14.6
(Mn/K) ×10^4	6.83	7.57	1.36	0.894	39.9	4.17	1.09	24.9
Na/K	12.2	10.8	1.9	1.55	41.8	7.54	2.92	41.5
P/K	257	345	61	47.5	1907	138	62.0	1017
Sr/K	0.89	1.44	0.25	0.073	8.05	0.535	0.090	4.04
Ca/Na	45.5	18.7	3.1	25.8	116	42.0	29.7	113
Cl/Na	0.213	0.255	0.042	0.027	1.58	0.159	0.028	0.583
K/Na	0.147	0.117	0.021	0.024	0.643	0.133	0.024	0.378
Mg/Na	0.456	0.300	0.049	0.129	1.87	0.401	0.156	1.01
(Mn/Na) ×10^4	0.726	0.688	0.115	0.129	3.62	0.543	0.179	0.282
P/Na	20.3	9.6	1.6	8.77	56.3	17.7	11.6	48.0
Sr/Na	0.760	0.691	0.114	0.055	3.39	0.561	0.131	2.93

M - arithmetic mean; SD – standard deviation; SEM – standard error of mean; Min – minimum value; Max – maximum value; Per. 0.025 – percentile with 0.025 level; Per. 0.975 – percentile with 0.975 level

Table 3. Some statistical parameters of 42 different ratios of Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the normal rib bone of both males and females, taken together

Ratio	M	SD	SEM	Min	Max	Med	P0.025	P0.975
(Cl/Ca)×10^3	5.38	2.76	0.31	0.699	13.6	4.69	1.30	12.8
(K/Ca) ×10^3	4.73	2.74	0.31	0.214	13.1	4.18	0.749	10.2
(Mg/Ca) ×10^2	1.10	0.32	0.04	0.355	2.15	1.16	0.431	1.61
(Mn/Ca) ×10^6	1.59	1.11	0.13	0.276	7.18	1.27	0.322	4.53
(Na/Ca) ×10^2	2.52	0.56	0.06	0.860	4.76	2.48	1.26	3.79
P/Ca	0.438	0.066	0.007	0.264	0.708	0.426	0.331	0.567
(Sr/Ca) ×10^3	1.52	0.85	0.10	0.140	5.88	1.38	0.332	2.92
Ca/P	2.33	0.35	0.04	1.41	3.79	2.35	1.76	3.02
(Cl/P) ×10^2	1.26	0.65	0.07	0.141	3.35	1.12	0.274	2.61
(K/P) ×10^2	1.12	0.66	0.08	0.052	3.17	1.02	0.178	2.53
(Mg/P) ×10^2	2.58	0.83	0.09	0.756	5.56	2.68	0.91	3.97
(Mn/P) ×10^6	3.61	2.40	0.28	0.627	12.7	2.98	0.785	11.1
(Na/P) ×10^2	5.90	1.70	0.19	1.77	13.6	5.79	2.17	8.40
(Sr/P) ×10^3	3.54	2.05	0.23	0.352	13.3	3.28	0.791	7.25
Ca/Mg	102	42.4	4.7	46.6	282	86.5	62.1	232
Cl/Mg	0.485	0.202	0.022	0.124	1.16	0.449	0.184	0.915
K/Mg	0.412	0.226	0.026	0.028	1.12	0.375	0.120	0.908
(Mn/Mg) ×10^4	1.72	1.93	0.22	0.212	12.8	1.25	0.254	6.29
Na/Mg	2.55	1.21	0.13	0.535	7.75	2.23	1.14	6.06
P/Mg	44.8	21.8	2.4	18.0	132	37.2	25.2	110
Sr/Mg	0.151	0.099	0.011	0.012	0.529	0.131	0.030	0.468
Ca/Cl	260	221	25	73.7	1431	213	77.9	770
K/Cl	0.867	0.403	0.046	0.051	1.94	0.815	0.325	1.91
Mg/Cl	2.47	1.19	0.13	0.860	8.09	2.23	1.09	5.45
(Mn/Cl) ×10^4	4.29	6.24	0.72	0.686	39.1	2.64	0.769	23.7
Na/Cl	6.49	5.84	0.65	0.634	36.7	5.05	2.12	20.1
P/Cl	116	110	12	29.9	710	89.7	38.4	365
Sr/Cl	0.381	0.462	0.052	0.042	3.78	0.283	0.064	1.28
Ca/K	384	576	66	76.4	4662	240	98.3	1342
Cl/K	1.62	2.18	0.25	0.516	19.6	1.23	0.523	3.10
Mg/K	3.63	4.21	0.48	0.892	36.3	2.67	1.10	8.31
(Mn/K) ×10^4	4.73	5.48	0.65	0.541	39.9	3.46	0.841	16.3
Na/K	8.36	8.12	0.93	1.55	41.8	5.77	2.56	32.0
P/K	166	241	28	31.6	1907	97.8	39.5	568
Sr/K	0.552	0.990	0.114	0.027	8.05	0.322	0.092	2.25
Ca/Na	42.4	14.3	1.6	21.0	116	40.4	26.4	79.6
Cl/Na	0.229	0.181	0.020	0.027	1.58	0.198	0.050	0.471
K/Na	0.189	0.106	0.012	0.024	0.643	0.173	0.032	0.391
Mg/Na	0.468	0.223	0.025	0.129	1.87	0.448	0.165	0.877
(Mn/Na) ×10^4	0.667	0.536	0.062	0.129	3.62	0.547	0.179	1.91
P/Na	18.7	7.6	0.84	7.37	56.3	17.3	11.9	46.1
Sr/Na	0.652	0.516	0.058	0.055	3.39	0.536	0.123	1.97

M - arithmetic mean; SD – standard deviation; SEM – standard error of mean; Min – minimum value; Max – maximum value; Per. 0.025 – percentile with 0.025 level; Per. 0.975 – percentile with 0.975 level

Table 4. Effect of age on mean values (M±SEM) of ratios of chemical element mass fractions in intact human rib bone (Student's *t*-test)

Ratio	Females			Males		
	15-35 year	36-55 year	*p*	15-35 year	36-55 year	*p*
$(Cl/Ca)\times10^3$	4.73±0.70	3.82±0.55	N.S.	5.70±0.41	6.87±0.60	N.S.
$(K/Ca)\times10^3$	3.33±0.40	3.05±0.62	N.S.	5.19±0.61	6.44±0.54	N.S.
$(Mg/Ca)\times10^2$	1.04±0.08	0.91±0.08	N.S.	1.24±0.05	1.19±0.06	N.S.
$(Mn/Ca)\times10^6$	1.48±0.15	1.74±0.39	N.S.	1.28±0.15	1.81±0.23	N.S.
$(Na/Ca)\times10^2$	2.37±0.12	2.44±0.15	N.S.	2.52±0.09	2.69±0.13	N.S.
P/Ca	0.43±0.01	0.46±0.02	N.S.	0.43±0.01	0.44±0.01	N.S.
$(Sr/Ca)\times10^3$	1.76±0.31	1.44±0.15	N.S.	1.33±0.15	1.56±0.14	N.S.
Ca/P	2.36±0.05	2.26±0.02	N.S.	2.36±0.06	2.34±0.07	N.S.
$(Cl/P)\times10^2$	1.11±0.17	0.90±0.15	N.S.	1.34±0.09	1.59±0.13	N.S.
$(K/P)\times10^2$	0.79±0.10	0.73±0.16	N.S.	1.23±0.15	1.51±0.13	N.S.
$(Mg/P)\times10^2$	2.46±0.18	2.12±0.26	N.S.	2.92±0.12	2.74±0.13	N.S.
$(Mn/P)\times10^6$	3.52±0.39	3.71±0.76	N.S.	2.97±0.33	4.15±0.58	N.S.
$(Na/P)\times10^2$	5.55±0.28	5.58±0.48	N.S.	5.99±0.30	6.36±0.41	N.S.
$(Sr/P)\times10^3$	4.16±0.72	3.28±0.44	N.S.	3.05±0.31	3.70±0.34	N.S.
Ca/Mg	108±10	130±14	N.S.	83.4±3.6	90.1±5.5	N.S.
Cl/Mg	0.43±0.04	0.41±0.04	N.S.	0.47±0.04	0.59±0.05	N.S.
K/Mg	0.31±0.03	0.30±0.05	N.S.	0.43±0.05	0.55±0.04	N.S.
$(Mn/Mg)\times10^4$	1.54±0.21	2.66±0.79	N.S.	1.04±0.12	1.63±0.23	≤0.05
Na/Mg	2.58±0.29	3.22±0.43	N.S.	2.11±0.12	2.37±0.13	N.S.
P/Mg	46.1±4.9	60.8±7.4	N.S.	35.7±1.7	39.0±2.7	N.S.
Sr/Mg	0.18±0.03	0.18±0.03	N.S.	0.11±0.01	0.14±0.02	N.S.
Ca/Cl	331±74	381±64	N.S.	192±12	167±12	N.S.
K/Cl	0.78±0.07	0.67±0.08	N.S.	0.91±0.09	1.01±0.10	N.S.
Mg/Cl	2.81±0.37	2.89±0.30	N.S.	2.38±0.18	1.94±0.17	N.S.
$(Mn/Cl)\times10^4$	4.56±1.04	7.59±2.5	N.S.	2.35±0.31	2.84±0.33	N.S.
Na/Cl	8.04±1.9	9.56±1.8	N.S.	4.73±0.28	4.35±0.28	N.S.
P/Cl	143±34	181±35	N.S.	82.0±5.4	72.7±6.1	N.S.
Sr/Cl	0.56±0.20	0.52±0.09	N.S.	0.25±0.03	0.24±0.03	N.S.
Ca/K	443±90	797±313	N.S.	260±38	199±27	N.S.
Cl/K	1.48±0.14	2.87±1.3	N.S.	1.34±0.16	1.21±0.11	N.S.
Mg/K	3.81±0.40	6.50±2.4	N.S.	3.07±0.34	2.26±0.32	N.S.
$(Mn/K)\times10^4$	6.10±1.2	7.72±2.7	N.S.	2.86±0.38	3.33±0.47	N.S.
Na/K	10.7±2.3	14.1±3.2	N.S.	6.24±0.87	5.02±0.57	N.S.
P/K	192±42	342±128	N.S.	112±17	86.8±12.1	N.S.
Sr/K	0.68±0.15	1.15±0.55	N.S.	0.33±0.06	0.28±0.05	N.S.
Ca/Na	45.6±4.3	45.4±4.5	N.S.	41.1±2.1	38.6±1.5	N.S.
Cl/Na	0.25±0.08	0.18±0.03	N.S.	0.23±0.01	0.26±0.02	N.S.
K/Na	0.16±0.03	0.13±0.02	N.S.	0.20±0.02	0.24±0.02	N.S.
Mg/Na	0.50±0.09	0.42±0.05	N.S.	0.51±0.03	0.45±0.02	N.S.
$(Mn/Na)\times10^4$	0.71±0.14	0.74±0.18	N.S.	0.51±0.06	0.71±0.09	N.S.
P/Na	19.3±1.7	21.3±2.6	N.S.	17.8±1.2	17.0±1.0	N.S.
Sr/Na	0.86±0.21	0.67±0.11	N.S.	0.54±0.06	0.58±0.06	N.S.

M – Arithmetical mean, SEM – standard error of mean, N.S. – non significant

Table 5. Effect of gender on mean values (M±SEM) of ratios of chemical element mass fractions in intact human rib bone (Student's *t*-test)

Ratio	Females	Males	*p*
(Cl/Ca)×10^3	4.26±0.44	6.32±0.38	≤0.01
(K/Ca) ×10^3	3.21±0.35	5.84±0.41	≤0.001
(Mg/Ca) ×10^2	0.98±0.06	1.21±0.04	≤0.01
(Mn/Ca) ×10^6	1.62±0.22	1.56±0.14	N.S.
(Na/Ca) ×10^2	2.41±0.10	2.61±0.08	N.S.
P/Ca	0.444±0.012	0.433±0.009	N.S.
(Sr/Ca) ×10^3	1.60±0.17	1.45±0.10	N.S.
Ca/P	2.31±0.06	2.35±0.05	N.S.
(Cl/P) ×10^2	1.00±0.11	1.47±0.08	≤0.001
(K/P) ×10^2	0.76±0.09	1.38±0.10	≤0.001
(Mg/P) ×10^2	2.29±0.16	2.82±0.09	≤0.01
(Mn/P) ×10^6	3.62±0.43	3.59±0.35	N.S.
(Na/P) ×10^2	5.57±0.28	6.18±0.26	N.S.
(Sr/P) ×10^3	3.71±0.42	3.38±0.23	N.S.
Ca/Mg	119±9	86.9±3.3	≤0.01
Cl/Mg	0.423±0.029	0.537±0.031	≤0.01
K/Mg	0.302±0.028	0.493±0.036	≤0.001
(Mn/Mg) ×10^4	2.13±0.43	1.35±0.14	N.S.
Na/Mg	2.91±0.26	2.24±0.09	≤0.05
P/Mg	53.6±4.5	37.4±1.6	≤0.01
Sr/Mg	0.181±0.020	0.125±0.010	≤0.05
Ca/Cl	357±49	179±9	≤0.001
K/Cl	0.733±0.053	0.963±0.067	≤0.01
Mg/Cl	2.85±0.23	2.15±0.13	≤0.01
(Mn/Cl) ×10^4	6.16±1.44	2.61±0.23	≤0.05
Na/Cl	8.82±1.32	4.53±0.20	≤0.01
P/Cl	162±24	77.2±4.1	≤0.01
Sr/Cl	0.536±0.104	0.248±0.020	≤0.01
Ca/K	598±147	228±23	≤0.05
Cl/K	2.09±0.57	1.27±0.10	N.S.
Mg/K	4.98±1.06	2.65±0.24	≤0.05
(Mn/K) ×10^4	6.83±1.36	3.11±0.30	≤0.01
Na/K	12.2±1.9	5.60±0.52	≤0.01
P/K	257±61	99.0±10.3	≤0.05
Sr/K	0.888±0.250	0.303±0.036	≤0.05
Ca/Na	45.5±3.1	39.8±1.3	N.S.
Cl/Na	0.213±0.042	0.242±0.012	N.S.
K/Na	0.147±0.021	0.220±0.013	≤0.01
Mg/Na	0.456±0.049	0.478±0.020	N.S.
(Mn/Na) ×10^4	0.726±0.115	0.614±0.055	N.S.
P/Na	20.3±1.6	17.4±0.8	N.S.
Sr/Na	0.760±0.114	0.559±0.041	N.S.

M – Arithmetical mean, SEM – standard error of mean, N.S. – non significant

A statistically significant tendency of gender-related differences was detected for Cl/Ca, K/Ca, Mg/Ca, Cl/P, K/P, Mg/P, Ca/Mg, Cl/Mg, K/Mg, Na/Mg, P/Mg, Sr/Mg, Ca/Cl, K/Cl, Mg/Cl, Mn/Cl, Na/Cl, P/Cl, Sr/Cl, Ca/K, Mg/K, Mn/K, Na/K, P/K, Sr/K, and K/Na mass fractions ratios. No gender-related dependence in Ca/P ratio in human rib bone was found in previously published studies [5-7]. Any published data referring to gender dependence of ratios of other chemical element mass fractions in human rib bone was not found.

Table 6 depicts the inter-correlation calculations including all chemical elements identified by us. The positive inter-correlations of Ca mass fractions with Mg (p<0.001), Mn (p<0.01), Na (p<0.001), P (p<0.001), and Sr (p<0.05) mass fractions were found in normal human rib-bone tissue.

Table 6. Inter-correlations (r – coefficient of correlation) of Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the normal human rib bone

Element	Ca	Cl	K	Mg	Mn	Na	P	Sr
Ca	**1.00**	0.144	0.047	0.562[c]	0.305[b]	0.648[c]	0.813[c]	0.264[a]
Cl	0.144	**1.00**	0.525[c]	0.470[c]	0.348[b]	0.038	0.067	0.251[a]
K	0.047	0.525[c]	**1.00**	0.332[b]	0.352[b]	0.312[b]	-0.038	0.106
Mg	0.562[c]	0.470[c]	0.332[b]	**1.00**	0.084	0.308[b]	0.487[c]	0.261[a]
Mn	0.305[b]	0.348[b]	0.352[b]	0.084	**1.00**	0.297[b]	0.274[a]	0.102
Na	0.648[c]	0.038	0.312[b]	0.308[b]	0.297[b]	**1.00**	0.418[c]	0.123
P	0.813[c]	0.067	-0.038	0.487[c]	0.274[a]	0.418[c]	**1.00**	0.250[a]
Sr	0.264[a]	0.251[a]	0.106	0.261[a]	0.102	0.123	0.250[a]	**1.00**

Statistically significant difference: [a] - $p \leq 0.05$, [b] - $p \leq 0.01$, [c] - $p \leq 0.001$.

Conclusions

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 Cl/Ca, K/Ca, Mg/Ca, Mn/Ca, Na/Ca, P/Ca, Sr/Ca, Ca/P, Cl/P, K/P, Mg/P, Mn/P, Na/P, Sr/P, Ca/Mg, Cl/Mg, Mn/Mg, Na/Mg, P/Mg, Sr/Mg, Ca/Cl, K/Cl, Mg/Cl, Mn/Cl, Na/Cl, P/Cl, Sr/Cl, Ca/K, Cl/K, Mg/K, Mn/K, Na/K, P/K, Sr/K, Ca/Na, Cl/Na, K/Na, Mg/Na, Mn/Na, P/Na, and Sr/Na mass fraction ratios in the intact rib bone may serve as indicative normal values for residents of the Central European region of Russia.

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