RELATIONSHIP BETWEEN Ca, Cl, K, Mg, Mn, Na, P, and Sr CONTENTS IN THE INTACT TRABECULAR BONE OF HUMAN ILIAC CREST INVESTIGATED BY NAA

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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 was to determine the Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the intact trabecular bone of human iliac crest 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. The third aim was to estimate the inter correlations between Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the intact trabecular bone of iliac crest.

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

Experimental

Iliac crest samples were obtained at postmortems from intact cadavers (38 female and 46 male, 15–55 years old) within 24 h of death. The 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 trabecular bone sample weighing about 50–100 mg. The

samples for INAA-SLR were sealed separately in thin polyethylene film 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 mass fractions 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 the Microsoft Office Excel 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 iliac trabecular 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 iliac trabecular bone.[5-7] No published data referring to ratios of other chemical element mass fractions in human iliac trabecular bone was found.

To estimate the effect of age on the chemical element ratios in iliac trabecular 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 iliac trabecular 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 iliac trabecular 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 intact iliac trabecular bone of males

Ratio	M	SD	SEM	Min	Max	Med	P0.025	P0.975
$(Cl/Ca)\times10^3$	13.8	5.8	0.87	3.46	29.2	12.6	5.04	24.9
$(K/Ca)\times10^3$	19.5	11.6	1.8	1.90	48.6	16.4	4.42	47.6
$(Mg/Ca)\times10^2$	1.21	0.33	0.05	0.540	2.14	1.17	0.720	1.94
$(Mn/Ca)\times10^6$	2.24	1.23	0.19	0.885	6.44	2.06	0.983	5.70
$(Na/Ca)\times10^2$	3.39	0.63	0.09	2.22	5.10	3.28	2.50	4.90
P/Ca	0.488	0.080	0.012	0.382	0.816	0.476	0.395	0.711
$(Sr/Ca)\times10^3$	2.25	1.29	0.20	0.297	5.80	2.01	0.527	5.54
Ca/P	2.09	0.28	0.04	1.22	2.62	2.10	1.41	2.53
$(Cl/P)\times10^2$	2.85	1.19	0.18	0.724	6.72	2.66	1.20	5.04
$(K/P)\times10^2$	4.14	2.42	0.37	0.455	11.4	3.71	1.01	9.89
$(Mg/P)\times10^2$	2.49	0.62	0.09	1.30	4.50	2.41	1.51	3.48
$(Mn/P)\times10^6$	4.34	1.63	0.25	1.91	9.35	3.99	1.98	7.24
$(Na/P)\times10^2$	6.92	1.17	0.18	4.09	10.4	6.83	4.77	9.12
$(Sr/P)\times10^3$	4.68	2.74	0.43	0.777	13.4	4.50	0.888	12.7
Ca/Mg	86.9	22.6	3.4	46.7	140	83.5	51.5	129
Cl/Mg	1.21	0.53	0.08	0.313	2.89	1.13	0.562	2.82
K/Mg	1.59	0.81	0.12	0.348	3.67	1.44	0.358	3.09
$(Mn/Mg)\times10^4$	1.93	0.93	0.14	0.657	4.85	1.87	0.732	4.33
Na/Mg	2.88	0.65	0.10	1.65	4.11	2.93	1.84	3.93
P/Mg	41.9	9.7	1.5	22.2	67.0	41.5	28.7	60.6
Sr/Mg	0.181	0.091	0.014	0.0415	0.387	0.179	0.0430	0.347
Ca/Cl	82.1	36.2	5.5	26.5	201	78.6	34.7	171
K/Cl	1.38	0.60	0.09	0.381	3.02	1.31	0.429	2.65
Mg/Cl	0.936	0.332	0.050	0.346	1.80	0.878	0.354	1.60
$(Mn/Cl)\times10^4$	1.72	0.96	0.15	0.539	4.06	1.41	0.547	4.01
Na/Cl	2.57	0.87	0.13	1.21	4.93	2.37	1.36	4.59
P/Cl	39.6	16.3	2.5	13.5	83.8	36.8	15.3	80.0
Sr/Cl	0.182	0.124	0.019	0.0304	0.611	0.144	0.0441	0.450
Ca/K	64.8	37.1	5.7	17.3	169	50.4	20.6	162
Cl/K	0.837	0.404	0.062	0.250	2.36	0.761	0.333	1.83
Mg/K	0.754	0.417	0.064	0.227	2.08	0.676	0.274	1.83
$(Mn/K)\times10^4$	1.31	0.78	0.12	0.377	3.33	1.10	0.409	3.03
Na/K	2.02	0.92	0.14	0.744	4.57	1.74	0.788	4.32
P/K	29.3	14.5	2.3	8.80	69.4	24.7	10.1	55.0
Sr/K	0.128	0.077	0.012	0.0243	0.305	0.124	0.0261	0.283
Ca/Na	30.4	5.5	0.8	19.6	45.1	30.5	20.4	40.0
Cl/Na	0.407	0.137	0.021	0.0978	0.736	0.408	0.152	0.689
K/Na	0.539	0.254	0.039	0.0856	1.13	0.526	0.150	1.09
Mg/Na	0.356	0.088	0.013	0.154	0.547	0.338	0.244	0.510
$(Mn/Na)\times10^4$	0.668	0.358	0.055	0.258	1.79	0.583	0.293	1.71
P/Na	14.3	2.2	0.3	8.21	18.8	14.5	9.69	18.7
Sr/Na	0.660	0.345	0.053	0.113	1.56	0.624	0.147	1.53

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 intact iliac trabecular bone of females

Ratio	M	SD	SEM	Min	Max	Med	P0.025	P0.975
$(Cl/Ca)\times10^3$	12.3	5.9	1.1	5.89	27.9	10.6	6.31	27.7
$(K/Ca)\times10^3$	16.5	6.8	1.4	3.35	31.0	15.6	5.52	28.7
$(Mg/Ca)\times10^2$	1.28	0.25	0.05	0.777	1.72	1.30	0.872	1.70
$(Mn/Ca)\times10^6$	1.98	0.65	0.14	0.958	3.55	1.99	0.962	3.12
$(Na/Ca)\times10^2$	3.44	0.74	0.14	2.47	5.43	3.23	2.48	5.07
P/Ca	0.500	0.066	0.012	0.348	0.685	0.501	0.391	0.615
$(Sr/Ca)\times10^3$	2.04	0.77	0.16	0.444	3.78	1.96	0.755	3.48
Ca/P	2.03	0.27	0.05	1.46	2.87	2.00	1.64	2.57
$(Cl/P)\times10^2$	2.49	1.21	0.23	1.06	5.81	2.08	1.06	5.41
$(K/P)\times10^2$	3.42	1.68	0.34	0.615	7.77	3.01	0.986	7.16
$(Mg/P)\times10^2$	2.60	0.59	0.11	1.49	4.12	2.67	1.61	3.67
$(Mn/P)\times10^6$	4.25	1.70	0.36	2.04	9.27	3.72	2.14	8.06
$(Na/P)\times10^2$	6.60	1.02	0.20	4.84	8.38	6.73	4.88	8.29
$(Sr/P)\times10^3$	4.07	1.41	0.29	0.923	6.93	4.00	1.23	6.40
Ca/Mg	77.9	15.8	3.0	41.7	108	76.7	52.5	107
Cl/Mg	0.911	0.388	0.075	0.416	1.90	0.838	0.418	1.90
K/Mg	1.28	0.57	0.11	0.315	2.51	1.24	0.495	2.47
$(Mn/Mg)\times10^4$	1.59	0.70	0.15	0.754	2.98	1.45	0.792	2.97
Na/Mg	2.72	0.80	0.15	1.61	4.97	2.49	1.65	4.43
P/Mg	39.8	10.4	2.0	20.3	67.0	37.4	23.0	62.0
Sr/Mg	0.179	0.103	0.021	0.0301	0.464	0.152	0.0543	0.401
Ca/Cl	97.3	37.4	7.1	35.8	170	94.3	36.1	159
K/Cl	1.61	0.87	0.17	0.439	3.80	1.39	0.530	3.34
Mg/Cl	1.26	0.53	0.10	0.475	2.41	1.18	0.509	2.39
$(Mn/Cl)\times10^4$	2.02	1.22	0.25	0.534	4.81	1.83	0.583	4.58
Na/Cl	3.25	1.23	0.23	1.29	5.93	3.11	1.31	5.70
P/Cl	48.9	21.1	4.0	17.2	94.1	48.1	18.5	94.1
Sr/Cl	0.229	0.150	0.031	0.0159	0.681	0.204	0.0456	0.574
Ca/K	65.3	28.1	5.6	16.5	144	62.7	25.9	124
Cl/K	0.830	0.491	0.096	0.263	2.28	0.720	0.302	1.92
Mg/K	0.859	0.371	0.074	0.248	1.63	0.721	0.338	1.57
$(Mn/K)\times10^4$	1.13	0.56	0.12	0.226	2.15	1.06	0.311	2.10
Na/K	2.10	0.78	0.16	0.896	3.35	1.90	0.977	3.29
P/K	33.3	16.7	3.3	7.74	81.1	32.4	10.8	69.3
Sr/K	0.138	0.088	0.018	0.0207	0.335	0.118	0.0226	0.313
Ca/Na	30.3	5.9	1.1	18.4	40.5	30.9	19.8	40.3
Cl/Na	0.359	0.156	0.029	0.169	0.777	0.322	0.175	0.763
K/Na	0.520	0.244	0.048	0.109	1.12	0.498	0.152	1.02
Mg/Na	0.396	0.105	0.020	0.201	0.621	0.402	0.227	0.606
$(Mn/Na)\times10^4$	0.622	0.271	0.057	0.244	1.33	0.570	0.248	1.27
P/Na	15.0	3.0	0.6	8.64	20.7	14.7	8.79	20.5
Sr/Na	0.650	0.297	0.061	0.124	1.23	0.563	0.207	1.22

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 intact iliac trabecular bone of males and females, taken together

Ratio	M	SD	SEM	Min	Max	Med	P0.025	P0.975
$(Cl/Ca)\times10^3$	13.2	5.8	0.7	3.46	29.2	12.2	5.60	27.6
$(K/Ca)\times10^3$	18.4	10.2	1.2	1.90	48.6	15.9	4.02	47.3
$(Mg/Ca)\times10^2$	1.24	0.30	0.04	0.540	2.14	1.23	0.759	1.78
$(Mn/Ca)\times10^6$	2.16	1.07	0.13	0.885	6.44	2.02	0.963	5.29
$(Na/Ca)\times10^2$	3.41	0.67	0.08	2.22	5.43	3.25	2.48	4.97
P/Ca	0.493	0.075	0.009	0.348	0.816	0.479	0.391	0.692
$(Sr/Ca)\times10^3$	2.18	1.13	0.14	0.297	5.80	1.97	0.492	5.05
Ca/P	2.07	0.28	0.03	1.22	2.87	2.09	1.45	2.56
$(Cl/P)\times10^2$	2.71	1.20	0.14	0.724	6.72	2.60	1.06	5.35
$(K/P)\times10^2$	3.88	2.20	0.26	0.455	11.4	3.55	0.884	9.86
$(Mg/P)\times10^2$	2.53	0.61	0.07	1.30	4.50	2.51	1.49	3.65
$(Mn/P)\times10^6$	4.31	1.64	0.21	1.91	9.35	3.97	2.02	8.10
$(Na/P)\times10^2$	6.80	1.12	0.13	4.09	10.4	6.77	4.81	9.01
$(Sr/P)\times10^3$	4.46	2.35	0.29	0.777	13.4	4.11	0.908	10.6
Ca/Mg	83.5	20.6	2.4	41.7	140	80.0	49.9	128
Cl/Mg	1.09	0.50	0.06	0.313	2.89	0.982	0.418	2.43
K/Mg	1.48	0.74	0.09	0.315	3.67	1.38	0.350	3.08
$(Mn/Mg)\times10^4$	1.82	0.87	0.11	0.657	4.85	1.75	0.743	3.92
Na/Mg	2.82	0.71	0.08	1.61	4.97	2.69	1.67	4.12
P/Mg	41.1	10.0	1.2	20.3	67.0	39.0	23.8	62.2
Sr/Mg	0.180	0.095	0.012	0.0301	0.464	0.174	0.0423	0.367
Ca/Cl	88.0	37.1	4.4	26.5	201	80.7	35.5	170
K/Cl	1.47	0.72	0.09	0.381	3.80	1.31	0.434	3.04
Mg/Cl	1.06	0.45	0.05	0.346	2.41	0.965	0.416	2.12
$(Mn/Cl)\times10^4$	1.83	1.06	0.13	0.534	4.81	1.58	0.544	4.20
Na/Cl	2.84	1.07	0.13	1.21	5.93	2.55	1.31	5.10
P/Cl	43.1	18.8	2.2	13.5	94.1	38.4	16.7	86.1
Sr/Cl	0.199	0.134	0.016	0.0159	0.681	0.174	0.0392	0.535
Ca/K	65.0	33.8	4.1	16.5	169	60.8	19.5	151
Cl/K	0.834	0.435	0.052	0.250	2.36	0.758	0.307	1.98
Mg/K	0.793	0.401	0.049	0.227	2.08	0.695	0.264	1.74
$(Mn/K)\times10^4$	1.25	0.72	0.09	0.226	3.33	1.08	0.390	2.87
Na/K	2.05	0.87	0.11	0.744	4.57	1.82	0.847	3.76
P/K	30.8	15.4	1.89	7.74	81.1	27.0	9.61	64.4
Sr/K	0.131	0.080	0.010	0.0207	0.335	0.119	0.0242	0.300
Ca/Na	30.4	5.6	0.7	18.4	45.1	30.7	20.1	40.3
Cl/Na	0.388	0.145	0.017	0.0978	0.777	0.382	0.164	0.740
K/Na	0.532	0.249	0.030	0.0856	1.13	0.515	0.136	1.10
Mg/Na	0.372	0.096	0.011	0.154	0.621	0.365	0.231	0.576
$(Mn/Na)\times10^4$	0.652	0.329	0.041	0.244	1.79	0.578	0.256	1.60
P/Na	14.6	2.6	0.3	8.21	20.7	14.5	8.80	19.7
Sr/Na	0.656	0.326	0.040	0.113	1.56	0.598	0.138	1.34

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 the intact iliac trabecular bone (Student's *t*-test)

Ratio	Females			Males		
	15-35 year	36-55 year	*p*	15-35 year	36-55 year	*p*
$(Cl/Ca)\times10^3$	12.3±1.5	12.3±1.7	N.S.	12.9±1.0	14.7±1.42	N.S.
$(K/Ca)\times10^3$	14.8±1.9	18.1±1.9	N.S.	20.3±2.9	18.8±2.24	N.S.
$(Mg/Ca)\times10^2$	1.32±0.05	1.23±0.09	N.S.	1.26±0.09	1.17±0.05	N.S.
$(Mn/Ca)\times10^6$	2.00±0.23	1.97±0.17	N.S.	2.66±0.35	1.94±0.19	N.S.
$(Na/Ca)\times10^2$	3.25±0.21	3.66±0.17	N.S.	3.40±0.17	3.39±0.10	N.S.
P/Ca	0.506±0.018	0.493±0.018	N.S.	0.498±0.024	0.480±0.009	N.S.
$(Sr/Ca)\times10^3$	2.24±0.23	1.82±0.21	N.S.	1.97±0.36	2.49±0.21	N.S.
Ca/P	2.01±0.07	2.06±0.08	N.S.	2.08±0.08	2.10±0.04	N.S.
$(Cl/P)\times10^2$	2.47±0.30	2.52±0.36	N.S.	2.65±0.20	3.04±0.29	N.S.
$(K/P)\times10^2$	2.95±0.39	3.86±0.53	N.S.	4.07±0.51	4.19±0.53	N.S.
$(Mg/P)\times10^2$	2.63±0.10	2.56±0.23	N.S.	2.56±0.16	2.43±0.11	N.S.
$(Mn/P)\times10^6$	4.03±0.48	4.45±0.53	N.S.	4.82±0.35	4.00±0.35	N.S.
$(Na/P)\times10^2$	6.10±0.23	7.18±0.26	≤0.01	6.78±0.33	7.05±0.17	N.S.
$(Sr/P)\times10^3$	4.46±0.44	3.65±0.37	N.S.	4.17±0.80	5.13±0.39	N.S.
Ca/Mg	77.3±2.9	78.6±6.0	N.S.	87.1±6.0	86.8±3.7	N.S.
Cl/Mg	0.848±0.072	0.978±0.135	N.S.	1.07±0.07	1.33±0.13	N.S.
K/Mg	1.10±0.14	1.45±0.17	N.S.	1.61±0.20	1.57±0.16	N.S.
$(Mn/Mg)\times10^4$	1.56±0.21	1.63±0.23	N.S.	2.27±0.25	1.68±0.15	≤0.05
Na/Mg	2.47±0.12	3.01±0.28	N.S.	2.73±0.16	3.01±0.12	N.S.
P/Mg	38.9±1.6	40.9±3.9	N.S.	42.0±2.5	41.7±1.7	N.S.
Sr/Mg	0.171±0.022	0.187±0.037	N.S.	0.143±0.019	0.216±0.018	≤0.01
Ca/Cl	95.7±9.1	99.1±11.4	N.S.	85.1±5.3	79.3±9.3	N.S.
K/Cl	1.47±0.22	1.76±0.26	N.S.	1.43±0.16	1.33±0.11	N.S.
Mg/Cl	1.24±0.11	1.28±0.18	N.S.	1.03±0.07	0.853±0.066	N.S.
$(Mn/Cl)\times10^4$	1.80±0.36	2.21±0.37	N.S.	2.12±0.23	1.41±0.18	≤0.05
Na/Cl	2.98±0.26	3.56±0.40	N.S.	2.65±0.14	2.51±0.22	N.S.
P/Cl	48.9±5.5	48.8±6.0	N.S.	41.7±2.8	37.2±4.0	N.S.
Sr/Cl	0.223±0.038	0.234±0.050	N.S.	0.169±0.031	0.194±0.022	N.S.
Ca/K	66.3±8.0	64.4±8.2	N.S.	66.3±9.2	63.5±7.3	N.S.
Cl/K	0.890±0.145	0.770±0.131	N.S.	0.848±0.109	0.828±0.068	N.S.
Mg/K	0.893±0.101	0.828±0.111	N.S.	0.788±0.109	0.725±0.077	N.S.
$(Mn/K)\times10^4$	1.04±0.18	1.20±0.18	N.S.	1.50±0.22	1.16±0.14	N.S.
Na/K	2.08±0.23	2.12±0.23	N.S.	1.94±0.22	2.07±0.20	N.S.
P/K	33.7±4.5	32.9±5.1	N.S.	28.3±3.2	30.1±3.2	N.S.
Sr/K	0.135±0.023	0.140±0.029	N.S.	0.104±0.016	0.149±0.017	N.S.
Ca/Na	32.2±1.6	28.1±1.3	N.S.	30.7±1.4	30.2±1.0	N.S.
Cl/Na	0.378±0.037	0.338±0.047	N.S.	0.383±0.021	0.428±0.034	N.S.
K/Na	0.524±0.071	0.517±0.067	N.S.	0.574±0.065	0.507±0.047	N.S.
Mg/Na	0.420±0.022	0.369±0.034	N.S.	0.368±0.023	0.347±0.015	N.S.
$(Mn/Na)\times10^4$	0.610±0.083	0.634±0.080	N.S.	0.807±0.107	0.563±0.045	≤0.05
P/Na	16.2±0.8	13.8±0.7	≤0.05	14.3±0.6	14.4±03	N.S.
Sr/Na	0.723±0.092	0.577±0.076	N.S.	0.581±0.088	0.735±0.057	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 the intact iliac trabecular bone (Student's *t*-test)

Ratio	Females	Males	*p*
(Cl/Ca)×10^3	12.3±1.1	13.8±0.9	N.S.
(K/Ca) ×10^3	16.5±1.4	19.5±1.8	N.S.
(Mg/Ca) ×10^2	1.28±0.05	1.21±0.05	N.S.
(Mn/Ca) ×10^6	1.98±0.14	2.24±0.19	N.S.
(Na/Ca) ×10^2	3.44±0.14	3.39±0.09	N.S.
P/Ca	0.500±0.012	0.488±0.012	N.S.
(Sr/Ca) ×10^3	2.04±0.16	2.25±0.20	N.S.
Ca/P	2.03±0.05	2.09±0.04	N.S.
(Cl/P) ×10^2	2.49±0.23	2.85±0.18	N.S.
(K/P) ×10^2	3.42±0.34	4.14±0.37	N.S.
(Mg/P) ×10^2	2.60±0.11	2.49±0.09	N.S.
(Mn/P) ×10^6	4.25±0.36	4.34±0.25	N.S.
(Na/P) ×10^2	6.60±0.20	6.92±0.18	N.S.
(Sr/P) ×10^3	4.07±0.29	4.68±0.43	N.S.
Ca/Mg	77.9±3.0	86.9±3.4	N.S.
Cl/Mg	0.911±0.075	1.21±0.08	≤0.01
K/Mg	1.28±0.11	1.59±0.12	N.S.
(Mn/Mg) ×10^4	1.59±0.15	1.93±0.14	N.S.
Na/Mg	2.72±0.15	2.88±0.10	N.S.
P/Mg	39.8±2.0	41.9±1.5	N.S.
Sr/Mg	0.179±0.021	0.181±0.014	N.S.
Ca/Cl	97.3±7.1	82.1±5.5	N.S.
K/Cl	1.61±0.17	1.38±0.09	N.S.
Mg/Cl	1.26±0.10	0.936±0.050	≤0.01
(Mn/Cl) ×10^4	2.02±0.25	1.72±0.15	N.S.
Na/Cl	3.25±0.23	2.57±0.13	≤0.05
P/Cl	48.9±4.0	39.6±2.5	N.S.
Sr/Cl	0.229±0.031	0.182±0.019	N.S.
Ca/K	65.3±5.6	64.8±5.7	N.S.
Cl/K	0.830±0.096	0.837±0.062	N.S.
Mg/K	0.859±0.074	0.754±0.064	N.S.
(Mn/K) ×10^4	1.13±0.12	1.31±0.12	N.S.
Na/K	2.10±0.16	2.02±0.14	N.S.
P/K	33.3±3.3	29.3±2.3	N.S.
Sr/K	0.138±0.018	0.128±0.012	N.S.
Ca/Na	30.3±1.1	30.4±0.8	N.S.
Cl/Na	0.359±0.029	0.407±0.021	N.S.
K/Na	0.520±0.048	0.539±0.039	N.S.
Mg/Na	0.396±0.020	0.356±0.013	N.S.
(Mn/Na) ×10^4	0.622±0.057	0.668±0.055	N.S.
P/Na	15.0±0.6	14.3±0.3	N.S.
Sr/Na	0.650±0.061	0.660±0.053	N.S.

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

The statistically significant age-related increase of Na/P ratio was found in the intact iliac trabecular bone of females. An age-related decrease of Mn/Mg, Mn/Cl and Mn/Na ratio and, in contrast, an increase of Sr/Mg ratio was observed in the intact iliac trabecular bone of males.

A statistically significant gender-related differences was detected for Cl/Mg and Na/Cl, mass fractions ratios. No gender-related dependence in Ca/P ratio in human iliac trabecular bone was found in previously published studies.[5-7] No published data referring to gender-related changes of ratios of other chemical element mass fractions in human iliac trabecular bone were 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<0.05), Na (p<0.001), P (p<0.001), and Sr (p<0.05) mass fractions were found in iliac crest trabecular bone tissue.

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

Element	Ca	Cl	K	Mg	Mn	Na	P	Sr
Ca	**1.00**	0.125	-0.024	0.540^{c}	0.215^{a}	0.643^{c}	0.819^{c}	0.200^{a}
Cl	0.125	**1.00**	0.557^{c}	0.203^{a}	-0.074	0.272^{a}	0.119	0.118
K	-0.024	0.557^{c}	**1.00**	0.252^{a}	-0.079	0.274^{a}	0.084	0.137
Mg	0.540^{c}	0.203^{a}	.252^{a}	**1.00**	0.189	0.443^{c}	0.595^{c}	0.308^{b}
Mn	0.215^{a}	-0.074	-0.079	0.189	**1.00**	0.254^{a}	0.423^{c}	0.216^{a}
Na	0.643^{c}	0.272^{a}	0.274^{a}	0.443^{c}	0.254^{a}	**1.00**	0.632^{c}	0.426^{c}
P	0.819^{c}	0.119	0.084	0.595^{c}	0.423^{c}	0.632^{c}	**1.00**	0.340^{b}
Sr	0.200^{a}	0.118	0.137	0.308^{b}	0.216^{a}	0.426^{c}	0.340^{b}	**1.00**

Statistically significant difference: ^{a} - $p\leq0.05$, ^{b} - $p\leq0.01$, ^{c} - $p\leq0.001$.

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 chemical element mass fraction ratios in the intact iliac trabecular bone may serve as indicative normal values for residents of the Central European region of Russia.

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