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INAA of Ca, Cl, K, Mg, Mn, Na, P, and Sr contents in the human cortical and trabecular bone

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Concentration of Ca, Cl, K, Mg, Mn, Na, P, and Sr were determined by instrumental neutron activation analysis using short-lived radionuclides in intact cortical and trabecular bone of femoral neck and iliac crest of 81 relatively healthy 15–55 years old women ($n = 36$) and men ($n = 45$). In cortical bone the Ca, P, and Mg mass fractions in the femoral neck were statistically significantly higher, and Cl, K, and Na lower, than the values for the iliac crest. In trabecular bone the Cl, K, and Na mass fractions in the iliac crest were significantly higher, and Ca, P also higher, than the values for the femoral neck.

Introduction

The incidence of bone diseases such as osteoporosis increases with advancing age and the fractures are usually the result of only relatively minor trauma. They represent a significant social and medical problem in terms of treatment and rehabilitation. Like many other diseases, it is easier to prevent osteoporosis than to treat it. Under these circumstances, the reliable early diagnosis of a bone disorder is of particular importance.

It is known that the control of the mineral component providing bone strength is a good indicator to detect bone diseases like osteoporosis. The bones of the skeleton have different levels of mineralization and turnover rate. Thus, every effort is made to search for those bones or part of the skeleton that shows marked deterioration in very early stages of the disease. The upper extremity of the femur in humans is a particularly vulnerable section of the skeleton, being subject to fracture and necrosis and to destruction of its cartilage. The iliac crest is the only bone from which biopsies are commonly taken on patients. Despite this, there have been few studies of the changes occurring with age in the femoral neck and iliac crest.[1]

In this study, the effects of age and sex on Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in cortical and trabecular (cancellous) bone of femoral neck and iliac crest from 81 relatively healthy subjects (36 women and 45 men) aged between 15 and 55 years were determined. The aim was to obtain parameters characteristic of the iliac cortical bone under in situ condition. Such a requirement should avoid possible bone destruction in both sample preparation using the ashing method, and also the removal of bone fat, marrow, or organic components using different organic solvents. It is known that such procedures result in substantial losses of both trace and major elements.[2–5] For this study the non-destructive method of instrumental neutron activation analysis (INAA) was applied.

Experimental

Human bone samples were collected during the period June 1995 to March 1998 at the Obninsk Hospital Department of Forensic Medicine. Samples were obtained at necropsy from the right side within twenty-four hours after death. All subjects had died suddenly. A tool made of titanium and plastic was used to clear samples off soft tissues and blood and to cut cortical and trabecular samples of bone. They were then freeze dried until a constant mass was obtained. The IAEA and NIST reference materials (H-5 Animal Bone and SRM1486 Bone Meal) were used to estimate the precision and accuracy of the results.

A horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor was used to determine Ca, Cl, K, Mg, Mn, Na, P, and Sr by INAA using short-lived radionuclides. The neutron flux in the channel was $1.7\cdot10^{13}$ n·cm^{-2}·s^{-1}. Each sample was measured twice, 1 minute and 120 minutes after irradiation. The duration of the first and second measurements was 10 minutes and 20 minutes, respectively. A coaxial 98 cm^3 Ge(Li) detector and a spectrometric unit (NUC 8100), including a PC-coupled multichannel analyzer, were used for analysis. The spectrometric unit provided 2.9 keV resolution at the ^{60}Co 1332 keV line.

Data concerning the nuclear reactions, radionuclides, gamma-energies, conditions of the analysis and analytical quality control have previously been published.[6,7] Mean data for each element in the reference materials were within the certified 95% confidence interval, thereby indicating an acceptable accuracy of the measured results.[6,7]

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Results and discussion

In order to estimate the effect of age on the investigated parameters the subjects were subdivided into two groups, aged 15–35 years, and 36–55 years. Table 1 gives the comparison of data regarding Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fraction in the intact cortical and trabecular bone of femoral neck and iliac crest of healthy women and men in the two age groups. Similar data for the two age groups combined are shown in Table 2.

Table 3 shows the comparison of mean values of chemical element mass fraction in cortical and trabecular bone of femoral neck and iliac crest separately for each of the two age subgroups of women and men.

Results of comparison Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fraction in cortical and trabecular bone of femoral neck and iliac crest for whole groups of women and men and in common (without of gender differences) are shown in Table 4.

No statistically significant differences of chemical element contents in female cortical bone of femoral neck and iliac crest were detected in two age subgroups (Table 1). The only exception was Mg, for which mass fractions in femoral neck were higher (p<0.05 or better). More differences were found between male cortical bone of femoral neck and iliac crest, particularly among the older age group. Mass fractions of Ca, Mg, and P in male cortical bone of femoral neck was statistically significantly higher, while Cl and Na lower, than in cortical bone of iliac crest.

The K and Na concentration in female trabecular bone of iliac crest were statistically significantly higher than those of femoral neck. The same differences were found in male trabecular bone of the younger age group. Among the older persons the differences were more pronounced and Cl mass fractions were higher too.

For both age groups combined (Table 2) the composition of female cortical bone was again very similar in both femoral neck and iliac crest with the exception of Mg for which the femoral neck level was significantly (p<0.001) higher (Table 2). More differences were again evident between male cortical bone, namely higher mass fractions of Ca, Mg and P in femoral neck, and of Cl, K, Na and Sr in iliac crest.

The total (female and male) cortical bone of femoral neck was higher in Ca, Mg and P, and lower in Cl, K and Na than cortical bone of iliac crest. In contrast, the human trabecular bone of iliac crest was higher in Cl, K and Na than trabecular bone of femoral neck (Table 2).

The most statistically significant differences were found between the compositions of cortical and trabecular bone in both femoral neck and iliac crest of both age group of women and men (Table 3). The Ca, Mg, Na and P mass fractions of femoral cortical bone are nearly twice those of femoral trabecular bone without reference of gender (Table 4). The iliac cortical bone differed from iliac trabecular bone not only in high levels of Ca, Mg, Na and P mass fractions, but also in lower levels of K (Table 4). The male cortical bone was higher in Sr than trabecular bone, particularly in femur (Table 4). The total human (combined femoral neck and iliac crest of female and male) cortical bone contained more Ca, Mg, Na and P, and less K, than trabecular bone.

Our means for Ca, Cl, Mg, Na and P (Tables 1 to 4) agree well with medians of the mean values cited by other researchers for the femoral cortical bone.[4,5,8–15] All published data of major and trace element concentrations in iliac cortical bone[16–18] are based upon techniques in which the sample is subjected to a number of treatments in order to remove fat, marrow, blood or the whole organic matrix. Thus, the determined mass fractions of chemical elements are referred mainly to the mineral part of the bone and not to the intact bone. The samples investigated by us are really intact ones. This is the reason why our means of Ca and P mass fractions are 5–10%, and Mg 10–20%, lower than the published data.[16–18] Despite the differences in sample treatment, our results for Na and K concentrations agree well with medians of the mean values cited by other researchers for the iliac cortical bone.[16,18]

Treatment of samples is the reason why our means of Ca, Mg and P mass fractions of trabecular bone are 1.6–2.0 times lower than the median value of published data for fat and marrow-free bone samples.[14,18–26] Our results for these elements are in better agreement with data of untreated samples.[4,12] Our data of Na concentration agree well with the mean values cited by other researchers for iliac cancellous bone.[18,19] The Cl means found in the study are in 16 times higher than previously reported values for iliac trabecular bone,[18] but agree well with cited data for the femoral cancellous bone.[4,23] Our Mn means are more than 5 times lower than previously reported values for the femoral cancellous bone.[12] The Sr means found in the study are from 2.3 to 3.1 times higher than published values.[16] It is known that Sr in the bone depends greatly on dietary habits and biogeochemical peculiarities of the residence area.[18,19,26] This may be the cause for the high Sr content in the bone of Russians.

Table 1. Effect of age on mean values (M±S.E.M.) of chemical element mass fraction in the intact cortical and trabecular bone of human femoral neck [FN] or iliac crest [IC] (in dry weight basis)

Bone	Sex	FN or IC	Age, years	*n*	Ca, g/kg	Cl, mg/kg	K, mg/kg	Mg, mg/kg	Mn, mg/kg	Na, mg/kg	P, g/kg	Sr, mg/kg
Cortical	Female	FN	15–35	15	224±9	1180±100	613±62	3020±220	<0.43±0.04	5240±220	100±5	282±57
		IC	15–35	18	216±6	1380±110	655±68	2270±125	<0.31±0.02	5590±240	103±4	315±47
			p(*t*-test)		N.S.	N.S.	N.S.	≤0.01	–	N.S.	N.S.	N.S.
		FN	36–55	18	224±11	1410±130	608±113	2720±180	<0.50±0.07	4980±180	110±6	309±43
		IC	36–55	18	207±7	1190±125	648±86	2140±140	<0.40±0.05	5700±300	98.2±4.3	304±43
			p(*t*-test)		N.S.	N.S.	N.S.	≤0.05	–	≤0.05	N.S.	N.S.
	Male	FN	15–35	21	233±8	1090±110	665±66	2790±110	<0.38±0.03	4990±150	106±6	255±35
		IC	15–35	20	221±6	1480±110	852±84	2300±140	<0.40±0.03	5820±220	97.4±3.6	317±42
			p(*t*-test)		N.S.	≤0.05	N.S.	≤0.01	–	≤0.01	N.S.	N.S.
		FN	36–55	24	236±8	1040±120	597±61	2530±100	<0.43±0.03	5170±210	111±4	227±19
		IC	36–55	24	191±7	1460±100	806±82	1690±80	<0.34±0.03	5310±230	85.7±3.1	299±24
			p(*t*-test)		≤0.001	≤0.01	≤0.05	≤0.001	–	N.S.	≤0.01	≤0.05
Trabecular	Female	FN	15–35	16	128±8	1330±160	641±54	1740±115	<0.42±0.07	3600±210	60.9±4.7	296±56
		IC	15–35	15	142±8	1680±180	1330±170	1850±90	<0.34±0.05	4460±190	70.9±3.2	265±30
			p(*t*-test)		N.S.	N.S.	≤0.001	N.S.	–	≤0.01	N.S.	N.S.
		FN	36–55	18	114±6	1370±120	590±73	1625±110	<0.47±0.06	3200±150	58.0±3.7	268±54
		IC	36–55	14	120±5	1430±200	1350±170	1550±110	<0.32±0.04	4470±250	59.2±2.9	217±33
			p(*t*-test)		N.S.	N.S.	≤0.001	N.S.	–	≤0.001	N.S.	N.S.
	Male	FN	15–35	20	118±6	1210±110	684±92	1600±110	<0.27±0.04	3130±200	56.3±3.0	168±27
		IC	15–35	21	122±6	1490±60	1190±130	1510±105	<0.36±0.05	4060±210	56.3±3.0	197±38
			p(*t*-test)		N.S.	≤0.05	≤0.01	N.S.	–	≤0.01	N.S.	N.S.
		FN	36–55	24	101±4	910±80	537±95	1330±90	<0.33±0.05	2870±100	50.2±2.2	154±19
		IC	36–55	24	125±8	1810±180	1380±130	1410±80	<0.26±0.02	4110±220	50.2±2.2	265±34
			p(*t*-test)		≤0.05	≤0.001	≤0.001	N.S.	–	≤0.001	N.S.	≤0.01

N.S.: Not significant.

Table 2. Difference between mean values (M±S.E.M.) of chemical element mass fraction in the intact cortical bone of human femoral neck [FN] and iliac crest [IC] (in dry weight basis)

Bone	Sex	FN or IC	*n*	Ca, g/kg	Cl, mg/kg	K, mg/kg	Mg, mg/kg	Mn, mg/kg	Na, mg/kg	P, g/kg	Sr, mg/kg
Cortical	Female	FN	33	224±7	1300±90	610±60	2850±140	<0.46±0.04	5100±140	105±4	295±35
		IC	36	211±5	1290±85	651±54	2210±95	<0.36±0.03	5640±190	101±3	309±31
		p(*t*-test)		N.S.	N.S.	N.S.	≤0.001	–	≤0.05	N.S.	N.S.
	Male	FN	45	235±6	1070±80	630±45	2650±80	<0.41±0.02	5080±130	109±3	240±20
		IC	44	205±5	1470±70	826±58	1960±90	<0.37±0.02	5540±160	91.1±2.5	307±23
		p(*t*-test)		≤0.001	≤0.001	≤0.01	≤0.001	–	≤0.05	≤0.001	≤0.05
Trabecular	Female	FN	34	121±5	1350±95	612±47	1680±80	<0.45±0.05	3390±130	59.4±2.9	283±39
		IC	29	132±5	1560±130	1340±120	1700±80	<0.33±0.03	4470±150	65.2±2.4	240±22
		p(*t*-test)		N.S.	N.S.	≤0.001	N.S.	–	≤0.001	N.S.	N.S.
	Male	FN	44	109±4	1050±70	606±67	1455±70	<0.31±0.03	2990±110	53.0±1.9	161±16
		IC	45	124±5	1660±100	1300±90	1460±70	<0.30±0.03	4090±150	59.6±2.4	233±25
		p(*t*-test)		≤0.05	≤0.001	≤0.001	N.S.	–	≤0.001	≤0.05	≤0.05
Cortical	Female and male	FN	78	230±4	1170±60	620±40	2740±70	<0.43±0.02	5090±90	107±3	260±18
		IC	80	208±3	1390±55	746±41	2070±65	<0.36±0.02	5590±120	95.3±1.7	308±18
		p(*t*-test)		≤0.001	≤0.01	≤0.05	≤0.001	–	≤0.01	≤0.001	N.S.
Trabecular	Female and male	FN	78	114±3	1180±60	609±41	1550±50	<0.37±0.03	3160±90	56.2±1.7	212±20
		IC	74	127±4	1620±80	1310±70	1550±50	<0.32±0.02	4240±110	61.8±1.8	235±18
		p(*t*-test)		≤0.05	≤0.001	≤0.001	N.S.	–	≤0.001	≤0.05	N.S.

N.S.: Not significant.

Table 3. Comparison of mean values (M±S.E.M.) of chemical element mass fraction in the intact cortical and trabecular bone of human femoral neck [FN] or iliac crest [IC] of female and male aged 15–35 and 36–55 years (in dry weight basis)

Bone	Sex	Cortical or Trabecular	Age, years	*n*	Ca, g/kg	Cl, mg/kg	K, mg/kg	Mg, mg/kg	Mn, mg/kg	Na, mg/kg	P, g/kg	Sr, mg/kg
Femoral neck	Female	Cortical	15–35	15	224±9	1180±100	613±62	3020±220	<0.43±0.04	5240±220	100±5	282±57
		Trabecular	15–35	16	128±8	1330±160	641±54	1740±115	<0.42±0.07	3600±210	60.9±4.7	296±56
			p(*t*-test)		≤0.001	N.S.	N.S.	≤0.001	–	≤0.001	≤0.001	N.S.
		Cortical	36–55	18	224±11	1410±130	608±113	2720±180	<0.50±0.07	4980±180	110±6	309±43
		Trabecular	36–55	18	114±6	1370±120	590±73	1625±110	<0.47±0.06	3200±150	58.0±3.7	268±54
			p(*t*-test)		≤0.001	N.S.	N.S.	≤0.001	–	≤0.001	≤0.001	N.S.
	Male	Cortical	15–35	21	233±8	1090±110	665±66	2790±110	<0.38±0.03	4990±150	106±6	255±35
		Trabecular	15–35	20	118±6	1210±110	684±92	1600±110	<0.27±0.04	3130±200	56.3±3.0	168±27
			p(*t*-test)		≤0.001	N.S.	N.S.	≤0.001	–	≤0.001	≤0.001	N.S.
		Cortical	36–55	24	236±8	1040±120	597±61	2530±100	<0.43±0.03	5170±210	111±4	227±19
		Trabecular	36–55	24	101±4	910±80	537±95	1330±90	<0.33±0.05	2870±100	50.2±2.2	154±19
			p(*t*-test)		≤0.001	N.S.	N.S.	≤0.001	–	≤0.001	≤0.001	≤0.05
Iliac crest	Female	Cortical	15–35	18	216±6	1380±110	655±68	2270±125	<0.31±0.02	5590±240	103±4	315±47
		Trabecular	15–35	15	142±8	1680±180	1330±170	1850±90	<0.34±0.05	4460±190	70.9±3.2	265±30
			p(*t*-test)		≤0.001	N.S.	≤0.05	≤0.05	–	≤0.001	≤0.001	N.S.
		Cortical	36–55	18	207±7	1190±125	648±86	2140±140	<0.40±0.05	5700±300	98.2±4.3	304±43
		Trabecular	36–55	14	120±5	1430±200	1350±170	1550±110	<0.32±0.04	4470±250	59.2±2.9	217±33
			p(*t*-test)		≤0.001	N.S.	≤0.001	≤0.01	–	≤0.01	≤0.001	N.S.
	Male	Cortical	15–35	20	221±6	1480±110	852±84	2300±140	<0.40±0.03	5820±220	97.4±3.6	317±42
		Trabecular	15–35	21	122±6	1490±60	1190±130	1510±105	<0.36±0.05	4060±210	56.3±3.0	197±38
			p(*t*-test)		≤0.001	N.S.	≤0.05	≤0.001	–	≤0.001	≤0.001	≤0.05
		Cortical	36–55	24	191±7	1460±100	806±82	1690±80	<0.34±0.03	5310±230	85.7±3.1	299±24
		Trabecular	36–55	24	125±8	1810±180	1380±130	1410±80	<0.26±0.02	4110±220	50.2±2.2	265±34
			p(*t*-test)		≤0.001	N.S.	≤0.01	≤0.05	–	≤0.001	≤0.001	N.S.

N.S.: Not significant.

Table 4. Difference between mean values (M±S.E.M.) of chemical element mass fraction in the intact cortical and trabecular bone of human femoral neck and iliac crest (in dry weight basis)

Bone	Sex	Cortical or Trabecular	*n*	Ca, g/kg	Cl, mg/kg	K, mg/kg	Mg, mg/kg	Mn, mg/kg	Na, mg/kg	P, g/kg	Sr, mg/kg
Femoral neck	Female	Cortical	33	224±7	1300±90	610±60	2850±140	<0.46±0.04	5100±140	105±4	295±35
		Trabecular	34	121±5	1350±95	612±47	1680±80	<0.45±0.05	3390±130	59.4±2.9	283±39
		p(*t*-test)		≤0.001	N.S.	N.S.	≤0.001	–	≤0.001	≤0.001	N.S.
	Male	Cortical	45	235±6	1070±80	630±45	2650±80	<0.41±0.02	5080±130	109±3	240±20
		Trabecular	44	109±4	1050±70	606±67	1455±70	<0.31±0.03	2990±110	53.0±1.9	161±16
		p(*t*-test)		≤0.001	N.S.	N.S.	≤0.001	–	≤0.001	≤0.001	≤0.01
Iliac crest	Female	Cortical	36	211±5	1290±85	651±54	2210±95	<0.36±0.03	5640±190	101±3	309±31
		Trabecular	29	132±5	1560±130	1340±120	1700±80	<0.33±0.03	4470±150	65.2±2.4	240±22
		p(*t*-test)		≤0.001	N.S.	≤0.001	≤0.001	–	≤0.001	≤0.001	N.S.
	Male	Cortical	44	205±5	1470±70	826±58	1960±90	<0.37±0.02	5540±160	91.1±2.5	307±23
		Trabecular	45	124±5	1660±100	1300±90	1460±70	<0.30±0.03	4090±150	59.6±2.4	233±25
		p(*t*-test)		≤0.001	N.S.	≤0.001	≤0.001	–	≤0.001	≤0.001	N.S.
Femoral neck	Female and male	Cortical	78	230±4	1170±60	620±40	2740±70	<0.43±0.02	5090±90	107±3	260±18
		Trabecular	78	114±3	1180±60	609±41	1550±50	<0.37±0.03	3160±90	56.2±1.7	212±20
		p(*t*-test)		≤0.001	N.S.	N.S.	≤0.001	–	≤0.001	≤0.001	N.S.
Iliac crest	Female and male	Cortical	80	208±3	1390±55	746±41	2070±65	<0.36±0.02	5590±120	95.3±1.7	308±18
		Trabecular	74	127±4	1620±80	1310±70	1550±50	<0.32±0.02	4240±110	61.8±1.8	235±18
		p(*t*-test)		≤0.001	≤0.05	≤0.001	≤0.001	–	≤0.001	≤0.001	≤0.01
Femoral neck	Female and male	Cortical	158	219±3	1280±41	684±29	2400±72	<0.39±0.02	5340±85	101±1.9	284±19
and		Trabecular	152	120±3	1394±50	950±70	1550±35	<0.34±0.02	3686±115	58.9±1.4	223±15
Iliac crest		*p*(*t*-test)		≤0.001	N.S	≤0.001	≤0.001	–	≤0.001	≤0.001	N.S.

N.S.: Not significant.

No statistically significant age-dependence changes of Ca, Cl, K, Mg, Mn, Na, P and Sr mass fractions were detected in cortical femoral neck. The concentrations of elements such as Ca, Mg and P in the cortical iliac crest tend to decrease with age to a certain degree for both women and men. This tendency is more pronounced for men – mean differences between the 15–35 and 36–55 year age groups for Ca ~14%, Mg ~27%, and P ~12%. The concentrations of these elements in the trabecular bone of femoral neck tend to decrease with age in both women and men too. This tendency is more pronounced for men (Ca and Mg ~20%, P ~10%) than for women (Ca and Mg ~10%, P ~5%). However, statistically significant changes were found only for Ca in male bone. The statistically significant decrease of Ca, Mg and P contents in the iliac trabecular bone with age was found only for women (Ca ~15.5%, Mg ~16%, P ~16.5%). So, the iliac crest has to be selected as being more sensitive than the femoral neck for age-dependence control of Ca, Mg and P content – iliac trabecular bone for women and iliac cortical bone for men.

Conclusions

The INAA using short-lived radionuclides allow to determine in intact samples of human cortical and trabecular bone the concentrations of not less than 8 chemical elements: Ca, Cl, K, Mg, Mn, Na, P, and Sr. The method is simple, fast, multielemental, and non-destructive.

Cortical bone (the total female and male) of femoral neck is higher in Ca, Mg and P, and lower in Cl, K and Na concentration than cortical bone of iliac crest. The differences are more pronounced for male cortical bone. In contrast, the human trabecular bone of iliac crest is higher in Cl, K and Na than trabecular bone of femoral neck.

The total human (combined femoral neck and iliac crest of female and male) cortical bone contains more Ca, Mg, Na and P, and less K, than trabecular bone.

Age-dependence changes of mass fractions of chemical elements turn on kind of bone and gender. The iliac crest has to be selected as being more sensitive than the femoral neck for age-dependence control of Ca, Mg and P content – iliac trabecular bone for women and iliac cortical bone for men.

Treatment of samples in previous researches is the main reason of some disagreements of mass fraction means of our study with the median values of published data for fat and marrow-free bone samples. Especially it concerns trabecular bone.

Our data for Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in intact cortical and trabecular bone of femoral neck and iliac crest may serve as indicative normal values for residents of the Russian Central European region.

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