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XA9950553

Description of research carried out:

The main aim of the present study was to estimate the age dependence of mineral density for the different body regions in healthy population of the Russian European area (Moscow and surroundings). Besides, bone mineral density (BMD) was to be analyzed by two ways: both *in vivo* and *in vitro*. *In vivo* studies were carried out using the method of DEXA (LUNAR DPX-L Densitometer) at the Central Institute of Traumatology and Orthopedics (Moscow) on volunteers randomly chosen. Autopsy material received from Forensic Department of Municipal Hospital (Obninsk) was used for *in vitro* NAA analysis.

For August 31, 1996 bone mineral density was studied by DEXA on 94 healthy citizens of Moscow aged 15-55 (62 women and 32 men). The distribution of examined people among the age groups is given in Table 1.

Bone mineral density of femoral neck (right and left), spine (front view, L_2-L_4) and total body was measured in each studied person. All the examined people were also put questions by a doctor to fill in the appropriate questionnaire (an adapted version of WHO OSTEOPOROSIS PROJECT QUESTIONNAIRE, CASE CONTROL STUDY, JULY 15, 1993).

Autopsy samples were taken from 58 people died unexpectedly (accidents, murders, suicides, drowning, acute alcohol intoxication). The died had neither chronic nor systematic diseases. The distribution of the died within the age groups is shown in Table 2.

Autopsy samples included those of bone (femoral neck, iliac crest and rib), teeth and hair. Conventional surgical instruments were used for sampling. Samples were put in polyethylene containers, frozen and then transported to the analytical laboratory. Instruments made of titanium and plastic were used to clean samples from muscle and connective tissues. Bone sample edges interacting with generally used surgical instruments were cut with a titanium knife. Dental enamel was thoroughly wiped twice with alcohol-washed gauze wad to avoid contamination related to stomatologic pincers. After cleaning, samples of bone and teeth were weighed both by an ordinary way and when dipped into bidistilled water to estimate wet mass and sample density. Then samples were frozen and freeze dried. Right after lyophilization, samples were weighed again to estimated water content using sample dry and wet mass ratio. Dried samples were placed in polyethylene packages, sealed and kept until the analysis started.

INAA with short-lived radionuclides was used to study contents of bone major minerals (Ca, P, Mg, Sr) and electrolytes (Na, Cl, K). The majority of analyzed samples represented those of compact and trabecular femoral neck. For the short-lived INAA, about 100 mg bone samples were weighed, sealed in thin polyethylene film and put in a polyethylene ampoule. The ampoule containing Mn monitor as well was placed in a transport container to be irradiated in WWR-c reactor horizontal channel (Obninsk Branch of Karpov Institute of Physical Chemistry) equipped with pneumatic system. Neutron flux density in the channel was $1.7 \cdot 10^{13}$ $n \cdot cm^{-2} \cdot s^{-1}$. The time of irradiation, decay and spectrometric measurement was 1, 1 and 5÷10 min, respectively. 1.5÷2 hours after irradiation, samples were again measured for 10÷20 min. Contents of Ca, P, Mg and Cl were determined during the first set of measurement using the emitting intensities of ^{49}Ca, ^{28}Al, ^{27}Mg and ^{38}Cl, respectively, while contents of Na, K, Mn and Sr were assessed during the second set of measurement in accordance with emitting intensities of ^{24}Na, ^{42}K, ^{56}Mn and ^{87m}Sr, respectively.

To ensure measurements, NUC-8100 spectrometric system (Hungary) and 40 cm^3 Ge(Li) detector were used to provide about 4 KeV resolution at 1333 KeV line (^{60}Co). The element concentrations were calculated by a relative way comparing the intensities of analytical photopeaks in the samples with those in the standards. Used were standards

prepared from chemically pure compounds as well as synthetic standards of the Institute of Physics, Georgian Academy of Sciences, based on phenol-formaldehyde resin. The precision of the results was verified by simultaneous measurement of H-5 IAEA reference material (animal bone).

The study of bone trace elements was started using INAA with long-lived radionuclides. For this purpose, 50 samples weighing about 100 mg each were packed in pure aluminium foil and placed in a quartz ampoule which was irradiated in IRT reactor channel (Moscow Institute of Engineering Physics) with the flux of approximately 10^{13} $n \cdot cm^{-2} \cdot s^{-1}$. Since ^{32}P β-bremsstrahlung was the reason of high background, the optimum decay time of bone samples was 90÷100 days. Up-to-date spectrometric measurement must ensure estimation of Sc, Cr, Fe, Co, Zn, Se, Rb, Ag, Sb, Cs and Hg using radiation of ^{46}Sc, ^{51}Cr, ^{59}Fe, ^{60}Co, ^{65}Zn, ^{75}Se, ^{86}Rb, ^{110m}Ag, ^{124}Sb, ^{134}Cs and ^{203}Hg, respectively.

Results obtained

The results of estimating the age dependence of mineral density for the femoral neck, spine and total body of healthy women and men using DEXA are given in Tables 3 and 4.

The age changes in concentrations of major minerals and electrolytes found for compact and trabecular bones of healthy male femoral neck with regard for the wet and dry tissue calculations are represented in Tables 5-8.

Information about femoral neck water content depending on the age of healthy men is shown in Table 9.

Conclusion drawn

The number of performed observations and analyses is evidently insufficient to make any final decision, however some preliminary conclusions can be drawn.

In accordance with DEXA results, BMD maximum for the femoral neck, spine and total body of healthy women is found for 31-35 age period (Table 3), while that of healthy men is reached by 21-25 age period, i.e. 10 year earlier (Table 4).

Follow INAA results, the full-value mineralization for both compact and trabecular tissue of healthy male femoral neck takes place by 15-20 age period (Tables 5-8). Contents of

major minerals (Ca, P, Mg, Sr) in compact femoral neck have no changes up to the age of 55. For the age above 30, concentrations of trabecular major minerals have a tendency to decrease. This tendency is most distinct for the age above 50 (Tables 6 and 8).

The content of Na increases slightly with age in the compact bone of femoral neck while the content of Cl decreases. There is also some increase of Na/Cl ratio with age. Since Na and Cl are mainly the extracellular electrolytes, the increase of Na/Cl ratio is an evidence of possible Na storage in hydroxiapatite crystals which can rise with age. This is expressed to a lesser degree for the trabecular bone.

The content of K in the compact femoral neck has almost no changes for the age period of 15-55 while that in trabecular bone decreases greatly with age. This tendency becomes especially apparent by the age of 51-55 ($p<0.05$). Because K is an extracellular electrolyte, the concentration of this element may indicate the reduce of bone cellular component.

Femoral neck water content of healthy men decreases with age (Table 9). So, if water content averages (24.0±1.5)% for the age period of 15-25, it is only (17.8±1.4)% ($p<0.001$) for the age of 26-55. Thus, bone is found to be dehydrated with age. It is quite clear that the number of observations and analyses should be increased to obtain statistically more reliable results.

In 1997 BMD studies using DEXA are supposed to be continued for the healthy Moscow citizens so that each male and female age group included not less than 15 people. It is also planned the autopsy material to be further collected with subsequent INAA. In addition to INAA of major and trace elements in bone samples, tooth and hair samples will be analyzed either.

Beginning from 1997, the cleaners of the Chernobyl accident will be examined by DEXA at the Medical Radiological Research Centre of RAMS. In case any person has clearly expressed osteoporosis symptoms, he will be recommended to be subjected to the iliac crest biopsy with further morphological and biochemical analysis as well as INAA for the purpose of diagnosis verification.

Table 1

The number of examined healthy women and men, citizens of Moscow (for August 31, 1996)

The age range (years)	The number of examined people	
	Female	Male
15-20	6	5
21-25	6	8
26-30	8	3
31-35	8	4
36-40	10	7
41-45	8	4
46-50	9	0
51-55	7	1
Total	**62**	**32**

Table 2

The number of died people from whom autopsy samples of bone(femoral neck, iliac crest, rib), teeth and hair were taken(for August 31, 1996)

The age range (years)	Female	Male
15-20	5	3
21-25	1	4
26-30	2	5
31-35	2	6
36-40	4	6
41-45	-	6
46-50	2	5
51-55	1	6
Total	**17**	**41**

Table 3

Results (Mean±SD) of estimating bone mineral density (BMD, g/cm^2) by DEXA in healthy women, citizens of Moscow, depending on the age

Region of interest		Female age (years): Range and Mean±SD							
		15-20 18.0±1.2	21-25 23.2±1.5	26-30 27.5±0.9	31-35 33.7±1.6	36-40 38.2±1.1	41-45 42.0±1.2	46-50 48.3±1.3	51-55 54.5±0.6
Femoral neck	left	1.067±0.169	0.986±0.210	1.039±0.113	1.073±0.187	0.978±0.099	0.992±0.138	1.063±0.085	0.960±0.080
	right	1.045±0.144	1.081±0.194	1.029±0.123	1.113±0.150	0.960±0.086	1.025±0.143	1.082±0.125	0.908±0.086
Spine	L_1-L_4	1.150±0.176	1.197±0.194	1.252±0.118	1.258±0.140	1.155±0.148	1.235±0.086	1.232±0.173	0.926±0.056
Total body		1.128±0.104	1.159±0.122	1.205±0.094	1.221±0.099	1.174±0.091	1.208±0.054	1.236±0.082	1.083±0.060

Table 4

Results (Mean±SD) of estimating bone mineral density (BMD, g/cm^2) by DEXA in healthy men, citizens of Moscow, depending on the age

Region of interest		Male age (years): Range and Mean±SD							
		15-20 16.8±2.5	21-25 23.5±1.7	26-30 28.0±1.7	31-35 32.3±1.1	36-40 38.3±1.4	41-45 41.5±0.7	46-50 48.3±1.3	51-55 54.5±0.6
Femoral neck	left	1.080±0.129	1.284±0.147	1.125±0.001	1.088±0.074	1.107±0.128	0.949±0.049	-	1.153
	right	1.098±0.122	1.280±0.160	1.075±0.084	1.058±0.082	1.097±0.139	0.949±0.047	-	1.114
Spine	L_2-L_4	1.040±0.041	1.226±0.168	1.248±0.147	1.221±0.134	1.171±0.135	1.053±0.128	-	1.113
Total body		1.122±0.048	1.347±0.125	1.287±0.132	1.255±0.033	1.293±0.113	1.184±0.096	-	1.236

Table 5

Concentrations of Ca, Mg, P, Sr, Na, Cl and K in wet compact femoral neck of healthy men depending on the age (Mean±SD, relative units)

The age range (years)	n	Ca	Mg	P	Sr	Na	Cl	K
15-20	3	1.00±0.21	1.00±0.16	1.00±0.21	1.00±0.69	1.00±0.30	1.00±0.44	1.00±0.09
21-25	1	0.90	0.93	0.82	0.33	2.77	0.88	0.46
26-30	2	0.91±0.12	0.97±0.19	0.93±0.12	1.77±0.18	0.59±0.32	0.76±0.05	<1.07±0.15
31-35	6	1.07±0.23	1.00±0.29	1.06±0.22	0.51±0.33	1.25±0.40	0.94±0.29	<1.07±0.45
36-40	3	0.91±0.08	0.83±0.11	0.91±0.10	0.65±0.15	1.11±0.33	0.77±0.31	<0.84±0.20
41-45	6	1.02±0.11	0.89±0.15	0.99±0.12	0.82±0.32	1.08±0.16	0.79±0.32	<1.06±0.67
46-50	4	1.06±0.12	0.83±0.08	1.06±0.14	0.81±0.45	1.07±0.30	0.69±0.15	<1.08±0.70
51-55	5	0.99±0.11	0.96±0.20	0.96±0.13	0.69±0.40	2.01±1.67	0.72±0.35	<0.82±0.27

Table 6

Concentrations of Ca, Mg, P, Sr, Na, Cl and K in wet trabecular femoral neck of healthy men depending on the age (Mean±SD, relative units)

The age range (years)	n	Ca	Mg	P	Sr	Na	Cl	K
15-20	3	1.00±0.06	1.00±0.24	1.00±0.12	1.00±0.61	1.00±0.28	1.00±0.43	1.00±0.41
21-25	1	0.93	1.15	0.86	0.50	0.73	0.94	0.82
26-30	2	1.19±0.21	0.96±0.12	1.08±0.11	1.03±0.88	1.13±0.46	0.78±0.34	<0.64±0.40
31-35	6	0.84±0.15	0.78±0.16	0.81±0.13	0.48±0.29	0.86±0.18	0.85±0.17	<0.80±0.64
36-40	3	0.84±0.13	0.86±0.12	0.81±0.11	0.77±0.11	0.66±0.32	0.45±0.20	<0.44±0.14
41-45	6	0.92±0.26	0.90±0.34	0.95±0.20	1.31±0.66	0.76±0.28	0.75±0.22	<0.95±0.89
46-50	4	0.96±0.15	0.73±0.26	0.89±0.10	0.93±0.44	0.64±0.07	0.64±0.54	0.67±0.24
51-55	5	0.84±0.27	0.79±0.34	0.86±0.30	0.58±0.24	1.07±0.67	0.71±0.19	0.50±0.12

Table 7

Concentrations of Ca, Mg, P, Sr, Na, Cl and K in dry compact femoral neck of healthy men depending on the age (Mean±SD, relative units)

The age range (years)	n	Ca	Mg	P	Sr	Na	Cl	K
15-20	3	1.00±0.14	1.00±0.10	1.00±0.14	1.00±0.67	1.00±0.26	1.00±0.42	1.00±0.17
21-25	1	0.87	0.89	0.79	0.31	0.96	0.84	0.43
26-30	2	0.81±0.11	0.86±0.17	0.83±0.11	1.54±0.17	0.53±0.20	0.67±0.05	<0.94±0.12
31-35	6	0.97±0.16	0.90±0.22	0.97±0.15	0.45±0.36	1.13±0.34	0.85±0.24	<0.95±0.39
36-40	3	0.81±0.11	0.73±0.07	0.81±0.12	0.57±0.15	0.99±0.32	0.68±0.25	<0.73±0.17
41-45	6	0.99±0.13	0.86±0.10	0.95±0.13	0.73±0.20	1.09±0.16	0.75±0.22	<0.93±0.53
46-50	4	0.95±0.10	0.74±0.08	0.95±0.12	0.71±0.38	0.96±0.26	0.61±0.14	<0.96±0.62
51-55	5	0.92±0.09	0.88±0.15	0.90±0.10	0.64±0.42	1.82±1.44	0.66±0.30	<0.75±0.25

Table 8

Concentrations of Ca, Mg, P, Sr, Na, Cl and K in dry trabecular femoral neck of healthy men depending on the age (Mean±SD, relative units)

The age range (years)	n	Ca	Mg	P	Sr	Na	Cl	K
15-20	3	1.00±0.10	1.00±0.32	1.00±0.15	1.00±0.62	1.00±0.28	1.00±0.52	1.00±0.40
21-25	1	0.89	1.08	0.82	0.47	0.70	0.88	0.77
26-30	2	1.04±0.14	0.83±0.09	0.94±0.09	0.90±0.76	0.99±0.33	0.67±0.30	<0.57±0.33
31-35	6	0.76±0.09	0.69±0.10	0.73±0.08	0.44±0.28	0.79±0.18	0.77±0.20	<0.76±0.66
36-40	3	0.74±0.09	0.74±0.08	0.70±0.06	0.67±0.12	0.57±0.26	0.38±0.16	<0.40±0.11
41-45	6	0.88±0.14	0.83±0.23	0.90±0.11	1.21±0.48	0.74±0.27	0.72±0.23	<0.95±0.91
46-50	4	0.86±0.13	0.64±0.23	0.80±0.09	0.82±0.39	0.58±0.04	0.55±0.46	0.70±0.36
51-55	5	0.76±0.20	0.71±0.27	0.78±0.23	0.55±0.20	0.73±0.27	0.63±0.16	0.45±0.08

Table 9

Femoral neck water content (Mean±SD) of healthy men depending on the age

The age range (years)	15-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55
n	3	1	2	6	3	6	4	5
H2O, %	24.8±2.0	22	14.7±0.1	17.5±1.3	14.7±0.6	21.5±2.8	15.8±0.2	18.6±1.4