Journal of Trace Elements in Medicine and Biology 24 (2010) 1–6

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.de/jtemb

Applied methodology

The effect of age and gender on 38 chemical element contents in human iliac crest investigated by instrumental neutron activation analysis

Sofia Zaichick [a,b], Vladimir Zaichick [a,b,*]

[a] Northwestern University, Department of Microbiology and Immunology, 302 E. Superior s., Morton Building, Chicago, IL 60611, USA
[b] Radionuclide Diagnostics Department, Medical Radiological Research Centre, Russian Academy of Medical Sciences, Koroleva Street 4, Obninsk 249020, Kaluga Region, Russia

ARTICLE INFO

Article history:
Received 22 January 2009
Accepted 29 July 2009

Keywords:
Chemical elements
Iliac crest
Neutron activation analysis

ABSTRACT

Project: To understand the role of major, minor, and trace elements in the etiology of bone diseases including osteoporosis, it is necessary to determine the normal levels and age-related changes of bone chemical elements.
Procedure: The effect of age and gender on 38 chemical element contents in intact iliac crest of 84 apparently healthy 15–55 years old women ($n = 38$) and men ($n = 46$) was investigated by neutron activation analysis.
Results: Mean values (M ± SEM) for mass fraction (on dry weight basis) of Ca, Cl, Co, Fe, K, Mg, Mn, Na, P, Rb, Sr, and Zn for both female and male taken together were Ca – 169 ± 3 g/kg, Cl – 1490 ± 43 mg/kg, Co – 0.0073 ± 0.0024 mg/kg, Fe – 177 ± 24 mg/kg, K – 1820 ± 79 mg/kg, Mg – 1840 ± 48 mg/kg, Mn – 0.316 ± 0.013 mg/kg, Na – 4970 ± 87 mg/kg, P – 79.7 ± 1.5 g/kg, Rb – 1.89 ± 0.22 mg/kg, Sr – 312 ± 15 mg/kg, and Zn – 65.9 ± 3.4 mg/kg, respectively. The upper limit of mean contents of Cs, Eu, Hg, Sb, Sc, and Se were Cs ≤ 0.09 mg/kg, Eu ≤ 0.005 mg/kg, Hg ≤ 0.005 mg/kg, Sb ≤ 0.004 mg/kg, Sc ≤ 0.001 mg/kg, and Se ≤ 0.1 mg/kg, respectively. In all bone samples the contents of Ag, As, Au, Ba, Br, Cd, Ce, Cr, Gd, Hf, La, Lu, Nd, Sm, Ta, Tb, Th, U, Yb, and Zr were under detection limits.
Conclusions: The Ca, Mg, and P contents decrease with age, regardless of gender. Higher Ca, Mg, P, and Sr mass fractions as well as lower Fe content are typical of female iliac crest as compared to those in male bone.

Introduction

Osteoporosis represents a significant social and medical problem in terms of treatment and rehabilitation [1,2]. The disease causes a decrease in the bone mineral part and subsequent bone strength. The bone can be considered a mineralized protein complex consisting of calcium hydroxyapatite [$Ca_{10}(PO_4)_6 \cdot (OH)_2$], collagen, marrow, fat, non-collagen proteins, water, minor and trace elements also. Since bone is a biomaterial that is structurally adapted to different functions and loading situations in adults, the exact composition may vary depending on sex, age, type and site, and also with alterations known to occur in bone metabolic diseases [3].

Bone strength depends mainly on the level of mineralization and contents of Ca and P consequently. The importance of minor and trace elements in provision of the bone function and strength has yet to be sufficiently studied [4,5]. There are several reviews and texts regarding chemical element analysis of different human bones, using chemical techniques and instrumental methods [6–9]. However, the majority of these data are based on non-intact bones. In most cases, bone samples are treated with solvents in order to remove collagen, fat, marrow and then are ashed and acid digested. There is evidence that by these methods some amount of chemical elements is lost upon treatment [10–13]. In addition, by this way the measured chemical element mass fractions are referred to the mineral part of bone and not to the whole intact bone. Thus, to understand the role of major, minor and trace elements in the etiology and pathogenesis of bone diseases including osteoporosis, it is necessary first of all to determine the normal levels and age- and gender-related differences of chemical element contents in intact bone in a large-scale study.

In the present study the effect of age and gender on 38 chemical element mass fractions in iliac crest was analyzed with two objectives. One of the objectives was to perform measurements on iliac crest – only bone from which biopsies are commonly taken clinically on patients. The second objective was

* Corresponding author at: Medical Radiological Research Centre, Russian Academy of Medical Sciences, Koroleva Street-4, Obninsk 249020, Kaluga Region, Russia.
E-mail address: vezai@obninsk.com (V. Zaichick).

0946-672X/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jtemb.2009.07.002

to perform measurements on intact bone. Instrumental neutron activation analysis (INAA) as a non-destructive method allows investigating bone samples without any kind of treatment.

Material and methods

Samples

Anterior-superior parts from iliac crest (mainly from the right side) 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 °C until use. All subjects died suddenly due to automobile accident, falls, shootings, stabbing, hanging, acute alcohol poisoning, or hypothermia.

Sample preparation

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 longitudinal sections of the iliac crest weighing about 50–100 mg and containing cortical and trabecular parts in natural ratio. Two sub-samples were received from each iliac crest sample: one sub-sample for INAA using short-lived radionuclides (INAA-SLR) and the other one for INAA using long-lived radionuclides (INAA-LLR). The sub-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. The sub-samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

Standards and certified reference materials

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol–formaldehyde resins were used [14]. Corrected certified values of BSS element contents were reported by us before [15]. In addition to SSB, aliquots of commercial, chemically pure compounds were also used as standards. Ten CRM IAEA H-5 (Animal Bone) [16] 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.

Instrumentation and methods

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, SSB, 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 were reported by us before [17].

A vertical channel of nuclear reactor was applied to determine the contents of Ag, As, Au, Ba, Br, Cd, Ce, Co, Cr, Cs, Eu, Fe, Gd, Hf, Hg, La, Lu, Nd, Rb, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, U, Yb, Zn, and Zr by INAA-LLR. The quartz ampoule with bone samples, standards, and certified reference materials was soldered, positioned in a transport aluminum container and exposed to a 20-h neutron irradiation in a vertical channel with a neutron flux of 1.7×10^{12} n cm^{-2} s^{-1}. Seven days later irradiation samples were reweighed and repacked.

Each sample was measured twice, 7–10 and 40–60 days after irradiation. The second measurements were started 40 days after irradiation due to the high intensity of ^{32}P β-particles ($T_{1/2} = 14.3$ d). To reduce this ^{32}P background, a combined lead–cadmium–copper–aluminum filter was used. The duration of the first and second measurements was 10 min and 10 h, respectively. The gamma spectrometer included an HPGe detector (GEM 15180P, ORTEC, relative efficiency 15%) and an MCA combined

Table 1
INAA data of chemical elements of CRM IAEA H-5 Animal bone and SRM NIST 1486 bone meal (mg/kg on dry weight basis).

Element	CRM IAEA H-5		This work results	SRM NIST 1486		This work results
	Mean	Type	Mean ± SD	Mean	Type	Mean ± SD
Ag	–	–	<0.02 DL	–	–	<0.02 DL
As	0.013	N	<0.1 DL	0.006	N	<0.1 DL
Au	–	–	<0.01 DL	–	–	<0.01 DL
Ba	72 ± 28	C	<100 DL	–	–	270 DL
Br	3.6	N	<10 DL	–	–	<10 DL
Ca, g/kg	212	**C**	208 ± 2	266	C	271 ± 8
Cd	0.023	–	<2 DL	0,003	N	<2 DL
Ce	–	–	≤0.03	–	–	≤0.02
Cl	550	C	556 ± 16	–	–	253 ± 35
Co	0.25	N	0.56 ± 0.2-5	–	–	0.11 ± 0.02
Cr	2.56	N	<0.8 DL	–	–	≤0.9
Cs	–	–	≤0.05	–	–	≤0.06
Eu	–	–	≤0.015	–	–	≤0.02
Fe	79 ± 11	C	85 ± 17	99 ± 8	C	93 ± 11
Gd	–	–	<0.25 DL	–	–	<0.25 DL
Hf	–	–	≤0.04	–	–	≤0.04
Hg	0.008	N	≤0.01	–	–	≤0.01
K	680	C	620 ± 19	412	C	415 ± 63
La	–	–	<0.05 DL	–	–	<0.05 DL
Lu	–	–	<0.003 DL	–	–	<0.003 DL
Mg	3600	C	3620 ± 32	4660	C	4770 ± 18-0
Mn	–	N	0.85 ± 0.0-3	1	N	0.98 ± 0.0-8
Na	5000	C	4820 ± 40	5000	N	5270 ± 17-0
Nd	–	–	<0.1 DL	–	–	<0.1 DL
P, g/kg	102	C	94.3 ± 0,8	123	C	119 ± 3
Rb	1.07	N	≤1.0	–	–	≤0.9
Sb	0.024	N	≤0.02	–	–	≤0.02
Sc	–	–	<0.001 DL	–	–	<0.001 DL
Se	0.054	N	≤0.05	0.13	N	≤0.05
Sm	–	–	<0.01 DL	–	–	<0.01 DL
Sr	96 ± 17	C	103 ± 9	264 ± 7	C	263 ± 10
Ta	–	–	≤0.08	–	–	≤0.08
Tb	–	–	<0.03 DL	–	–	<0.03 DL
Th	–	–	<0.05 DL	–	–	<0.05 DL
U	–	–	<0.07 DL	–	–	<0.07 DL
Yb	–	–	<0.03 DL	–	–	<0.03 DL
Zn	89 ± 15	C	86 ± 7	147 ± 16	C	153 ± 29
Zr	–	–	<0.2 DL	–	–	<0.2 DL

Mean – arithmetical mean; SD – standard deviation; C – certified values; N – non-certified values; DL – detection limit.