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INAA of cortical and trabecular bone samples from animals

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Instrumental neutron activation analysis (INAA) was applied to determine Ba, Br, Ca, Cl, Fe, K, Mg, Mn, Na, P, Sr and Zn in bovine and porcine rib bones. Precise results were obtained in analyses of freeze-dried cortical and trabecular bones separately, and also of whole bone ashes. Cortical tissues presented higher concentrations of Ba, Ca, Mg, Mn, Na, P, Sr and Zn than those obtained in trabecular ones. Comparisons were also made between the results obtained for bovine and porcine rib bones.

Introduction

Trace element determinations in bones are of great importance for nutrition and prevention, controlling several diseases caused by mineral or trace element imbalance. The correlation of elements present in bones with chronic human diseases has been reported by several investigators,[1–3] and analyses of archaeologically recovered bones have been also assessed to determine ancient people's diet and health condition.[4–5]

There is also great interest in evaluating trace elements in different bone parts (trabecular and cortical) separately, since investigations on inorganic phases from cortical and trabecular bone have also shown several chemical differences.[6,7] Trabecular bone apatite has shown a smaller amount of carbonate ions and a lower Ca/P molar ratio than cortical bone apatite.[6]

Despite this importance, data about trace elements in different bone compartments are very scarce since it is difficult to obtain representative specimens for chemical analyses. Bones are commonly divided into two compartments: cortical (compact) and trabecular (spongy and porous, cancellous) based on their hardness, porosity, and content. However, not all bones can be strictly classified as cortical or trabecular since some types are intermediate in porosity and are difficult to classify. Besides, due to medico-legal implications, collecting samples from humans is generally a problem.

Several analytical techniques such as atomic absorption spectrometry (AAS),[2,8] inductively coupled plasma atomic emission spectrometry (ICP-AES),[2,9] ICP mass spectrometry (ICP-MS),[2] proton induced X-ray emission (PIXE),[7] proton induced prompt gamma-ray emission (PIGE)[7] and NAA[10–12] have been used for trace element determinations in human bones.

In this work, INAA was applied to trace element determinations in freeze-dried cortical and trabecular tissues and bone ash from bovine and porcine ribs. Comparisons were made between the results obtained in different tissues and also between the data obtained for bovine and porcine rib bones.

Experimental

Bovine and porcine rib bone samples and their treatment for the analyses

Twelve adult rib bones, being six from bovine species and six from porcine ones, were provided from the local butchery, wrapped in polyethylene foils and stored in a freezer at –19 °C until their treatment for the analyses. The ribs were cleaned free of connected soft tissues (periosteum) using a Ti knife and then cut into slices by a stainless steel saw. Purified water was used to remove blood, and the edges of the ribs were discarded. The trabecular part was removed and separated from the cortical tissue. This cortical tissue was broken up into small pieces. The trabecular and cortical tissues were then freeze-dried, and in this process, mean weight losses of 18.1% and 32.9% were obtained for cortical and trabecular tissues, respectively. For whole rib bone ash, the sample was calcinated during 10 hours at about 800 °C and then ground to obtain in a powder form. The mass fraction mean of ash in dry and wet bone was 0.39. Precautions were taken during this treatment step to avoid contamination. Handling of the samples was performed inside a class 100 laminar-flow hood and a Teflon spatula was used for transferring them. The water utilized was purified in a Millipore water purification system, and the samples were stored in Nalgene polyethylene vials.

Instrumental neutron activation analysis

Aliquots of about 100 to 150 mg of each sample were weighed in clean polyethylene involucres and irradiated in the IEA-R1 nuclear reactor with the synthetic standards of the elements. The synthetic standards were prepared by pippeting 50 μl of elemental

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solutions onto small sheets of Whatman No. 41 filter paper. These standard solutions containing one or more elements were prepared from certified standard solutions provided by SpexCertiprep Inc. The element qunatities varied from 0.6 to 1000 μg. In the case of P standard, 30 mg of ammonium dihydrogen phosphate, Puratonic, 99.998% purity from Alfa Aesar weighed directly in polyethylene involucre were utilized.

Two procedures were used for the irradiations. Two-minute irradiations at the rabbit pneumatic station with thermal neutron flux of $4.5 \cdot 10^{11}$ n·cm^{-2}·s^{-1} were performed to determine Ba, Ca, Cl, K, Mg, Mn, Na and Sr. Eight-hour irradiations under thermal neutron flux of about 10^{12} n·cm^{-2}·s^{-1} were used for Ba, Br, Ca, Fe, Na, Sr and Zn determinations. For 2-minute irradiations, the measurements were carried out after decay times of about 6 and 30 minutes. Two series of measurements were carried out after the 8-hour irradiations: the first was performed from 5–7 days after irradiation and the second one, after 15–20 days of decay. The gamma-activities of the samples and the element standards were measured using a Model GX2020 hyperpure Ge detector coupled to Model 1510 Integrated Signal Processor and MCA System 100, both from Canberra (USA). The counting system had a resolution of 0.90 keV for 121.97 keV gamma-ray of ^{57}Co and of 1.78 keV for 1331 keV of ^{60}Co. Short irradiations were also used for P determinations and the beta-activity of ^{32}P was measured in a Geiger-Müller detector after 10 days of decay time. The gamma-spectra were processed using VERSAO2 software, a new version of VISPECT2 software.[13] The radioisotopes were identified by half-lives and gamma-ray energies. A preliminary experiment was carried out to assure that there is no interference of other activated radionuclides of the samples in the counting. The sample was measured for different decay times and afterwards the determination of half-life was carried out for radioisotope identification. The comparative method was used for calculating the concentrations of elements in the samples. The radioisotopes used in theses analyses were: ^{139}Ba, ^{131}Ba, ^{82}Br, ^{49}Ca, ^{47}Ca, ^{38}Cl, ^{59}Fe, ^{42}K, ^{27}Mg, ^{56}Mn, ^{24}Na, ^{87m}Sr, ^{85}Sr and ^{65}Zn. Concentrations of Ba, Ca and Sr were obtained in both short and long irradiations. The mean value of the data of both irradiations were used.

Analysis of certified reference materials

To control the quality of the results, NIST SRM 1400 Bone Ash and NIST SRM 1486 Bone Meal certified reference materials were analyzed under the same conditions as for analyses of bone samples.

In order to express the concentrations of elements on a dry-mass basis, moisture was determined in a separate sub-sample (not taken for analysis) by drying at 105 °C for four hours. The following values (in percent) of weight loss were obtained: 0.40 for Bone Ash and 1.90 for Bone Meal. These values were used to correct the final results.

Results and discussion

Analytical results obtained for Bone Ash and Bone Meal certified reference materials are shown in Table 1. It can be observed that good results were found with relative standard deviations lower than 13.4% and percentage of relative errors lower than 12.7%. Standardized differences or z values[16] were obtained and they varied from 0.44 to 1.52, that is, $|z|<3$. This shows that our analytical results are within the interval of certified values at the significance level of 1%.

Table 2 presents means of the element concentrations obtained in the analyses of a bovine rib bone sample, and these results show a good reproducibility for most elements with relative standard deviations lower than the 13.5%. Br was not detected in whole rib bone ash, and the detection limit value was evaluated according to CURRIE.[17] Less precise results were obtained for Br in the cortical tissue and for Mn in the trabecular tissue. The gamma-ray photopeaks of ^{82}Br and ^{56}Mn were not pronounced, resulting in poor counting statistics. These findings indicated that the procedure adopted for bone sample treatment and homogenization was appropriate.

Analytical data obtained for bovine and porcine samples are presented in Tables 3 and 4, respectively. Statistical t-test, at the significance level of 5%, was applied to compare the results obtained for different sub-compartments of ribs and also the samples from two animal species analyzed. Concentrations of Ba, Ca, Mg, Mn, Na, P, Sr and Zn were higher in cortical tissues than those obtained in trabecular ones whereas these presented higher concentrations of Br, Fe and K. The high levels of Fe obtained in the trabecular part can be attributed to the presence of blood fluid in this tissue. Results obtained for whole rib bone ashes were within the data obtained for cortical and trabecular bones except the results obtained for Br and Cl. These elements presented lower concentrations in whole rib bone ashes than in cortical and trabecular bones, probably due to the loss of these elements during the calcination process.

Besides, most elements found in cortical, trabecular and calcinated total bones from bovine species were in the same magnitude as those found in corresponding tissues from porcine bones. The exceptions were Zn and Ba. Zn concentrations were higher in bovine bones than those in porcine ones. Ba was not detected in porcine rib bones and the detection limit value was evaluated.

Table 1. Concentrations of elements in reference materials NIST SRM 1400 Bone Ash and NIST SRM 1486 Bone Meal

Element	NIST SRM 1400 Bone Ash			NIST SRM 1486 Bone Meal		
	This work*		Reference 14	This work*		Reference 15
	M ± S.D.	R.S.D., %		M ± S.D.	R.S.D., %	
Ba, $\mu g \cdot g^{-1}$	249.3 ± 7.6	3.1	–	247 ± 15	5.9	–
Ca, %	35.6 ± 2.0	5.5	38.18 ± 0.13	25.6 ± 1.5	5.9	26.58 ± 0.24
Cl, $\mu g \cdot g^{-1}$	242 ± 10	4.2		197 ± 8	4.0	–
Fe, $\mu g \cdot g^{-1}$	660 ± 11	1.7	660 ± 27	86.4 ± 11.4	13.4	99 ± 8
Mg, %	0.65 ± 0.02	3.2	0.684 ± 0.013	0.45 ± 0.02	3.7	0.466 ± 0.017
Mn, $\mu g \cdot kg^{-1}$	16.5 ± 0.9	5.2	(17)**	1028 ± 108	10.5	(1000)
Na, $\mu g \cdot g^{-1}$	5800 ± 333	5.7	(6000)	5148 ± 209	4.1	(5000)
P, %	17.0 ± 1.2	7.2	17.91 ± 0.19	13.1 ± 1.3	9.8	12.30 ± 0.19
Sr, $\mu g \cdot g^{-1}$	248 ± 8	3.3	249 ± 7	270 ± 11	4.2	264 ± 8
Zn, $\mu g \cdot g^{-1}$	177 ± 9	4.8	181 ± 3	134.4 ± 14.6	10.8	147 ± 16

* M ± S.D.: Arithmetic mean and standard deviation in n determinations, ($3 \leq n \leq 7$); R.S.D., %: relative standard deviation.
** Number in parentheses indicates informative value.

Table 2. Means of the elemental concentrations obtained in a sample of bovine rib bone. All values, unless otherwise indicated, are given in $\mu g \cdot g^{-1}$, dried weight basis

Element	Cortical		Trabecular		Whole rib bone ash	
	M ± S.D.*	R.S.D., %	M ± S.D.	R.S.D., %	M ± S.D.	R.S.D., %
Ba	127.5 ± 1.6	1.2	118.4 ± 4.1	3.5	206.0 ± 4.2	2.0
Br	0.59 ± 0.08	13.6	1.73 ± 0.12	6.9	<0.2	–
Ca, %	21.8 ± 1.2	5.5	15.2 ± 1.6	10.5	33.8 ± 2.5	7.4
Cl	226.8 ± 3.1	1.4	816 ± 85	10.4	239 ± 3	1.3
Fe	<4.7**	–	121.0 ± 1.0	0.8	56.5 ± 3.5	6.2
K	815 ± 7	0.9	1589 ± 181	11.4	857 ± 46	5.3
Mg	4097 ± 79	1.9	3333 ± 403	12.1	6335 ± 206	3.3
Mn, $\mu g \cdot kg^{-1}$	436 ± 55	12.6	313 ± 65	20.7	383 ± 16	4.2
Na	5601 ± 116	2.1	4102 ± 561	13.7	8686 ± 554	6.4
P, %	8.5 ± 0.7	8.2	7.6 ± 0.6	7.9	15.9 ± 2.1	13.2
Sr	135.4 ± 0.6	0.4	242 ± 20	8.2	284 ± 13	4.6
Zn	53.6 ± 0.4	0.7	70.3 ± 2.3	3.3	96.4 ± 5.3	5.5

* M ± S.D.: Arithmetic mean and standard deviation in n determinations, ($4 \leq n \leq 7$); R.S.D, %: relative standard deviation.
** Detection limit.

Table 3. Concentrations of elements obtained in bovine rib bones. All values, unless otherwise indicated, are given in $\mu g \cdot g^{-1}$, wet weight basis

Element	Cortical (N=6)*		Trabecular, (N=6)		Whole rib bone ash (N=6)	
	M ± S.D.	Range	M ± S.D.	Range	M ± S.D.	Range
Ba	101 ± 41	38.9 – 167.7	48 ± 23	20.6 – 82.0	88 ± 33	53.3 – 140
Br	0.75 ± 0.38	0.28 – 1.31	1.91 ± 1.16	0.35 – 3.14	0.38 ± 0.14	0.10 – 0.40
Ca, %	18.3 ± 1.2	17.0 – 20.1	7.3 ± 1.8	5.2 – 9.7	14.7 ± 2.0	13.3 – 16.1
Cl	316 ± 103	134 – 415	749 ± 468	224.2 – 1395	394 ± 180	175 – 518
Fe	8.1 ± 0.8	4.7** – 8.7	137 ± 95	16.6 – 226.4	31.3 ± 18.5	8.0 – 51.5
K	550 ± 248	241 – 928	1062 ± 727	235** – 1735	1003 ± 480	369** – 1653
Mg	3140 ± 591	2272 – 3824	1515 ± 376	1070 – 1952	2916 ± 441	2544 – 3745
Mn, $\mu g \cdot kg^{-1}$	277 ± 79	197 – 388	157 ± 30	110 – 200	268 ± 66	149 – 335
Na	3879 ± 381	3210 – 4336	2304 ± 494	1422 – 2724	3814 ± 414	3264 – 4249
P, %	8.8 ± 1.1	7.2 – 10.6	3.4 ± 1.2	2.1 – 5.0	8.7 ± 1.9	6.31 – 11.4
Sr	193 ± 57	986 – 274.8	88 ± 44	43.1 – 145	193 ± 66	112 – 312
Zn	51.8 ± 5.4	44.9 – 60.4	36.7 ± 9.5	23.8 – 48.0	49.1 ± 6.6	41.2 – 56.1

* N: Number of samples.
** Detection limit.

Table 4. Concentrations of elements obtained in porcine rib bones. All values, unless otherwise indicated, are given in $\mu g \cdot g^{-1}$, wet weight basis

Element	Cortical (N=6)*		Trabecular (N=6)		Whole rib bone ash (N=6)	
	M ± S.D.	Range	M ± S.D.	Range	M ± S.D.	Range
Ba	<32.5	–	<15.7	–	<58	–
Br	0.37 ± 0.09	0.10** – 0.48	0.89 ± 0.55	0.34 – 1.84	0.13 ± 0.08	0.10** – 0.18
Ca, %	16.5 ± 1.2	15.3 – 18.2	7.8 ± 1.7	5.5 – 9.1	10.3 ± 1.7	8.2 – 12.5
Cl	178 ± 60	98.3 – 229	554 ± 240	195 – 636	129 ± 36	89.8 – 180.8
Fe	<9.3	–	61 ± 7	54.1 – 70.0	30 ± 6	23.7 – 30.9
K	643 ± 268	314 – 991	1110 ± 465	584 – 1820	606 ± 336	314 – 1157
Mg	3563 ± 358	3250 – 4172	1613 ± 325	1167 – 1849	2352 ± 455	1866 – 3090
Mn, $\mu g \cdot kg^{-1}$	326 ± 88	256.9 – 486.4	196 ± 21	166 – 220	230 ± 26	197.8 – 258.4
Na	3639 ± 218	3307 – 3913	2056 ± 387	1561 – 2464	2819 ± 461	2335 – 3130
P, %	7.1 ± 1.5	5.60 – 9.2	4.2 ± 0.9	2.94 – 5.38	6.5 ± 0.8	5.58 – 7.55
Sr	216 ± 115	62.5 – 348	118 ± 60	50.2 – 192.3	166 ± 70	69.5 – 213.6
Zn	119 ± 17	99.0 – 1419	64.4 ± 14	51.3 – 85.0	77 ± 23	57.8 – 121.8

* N: Number of samples.
** Detection limit.

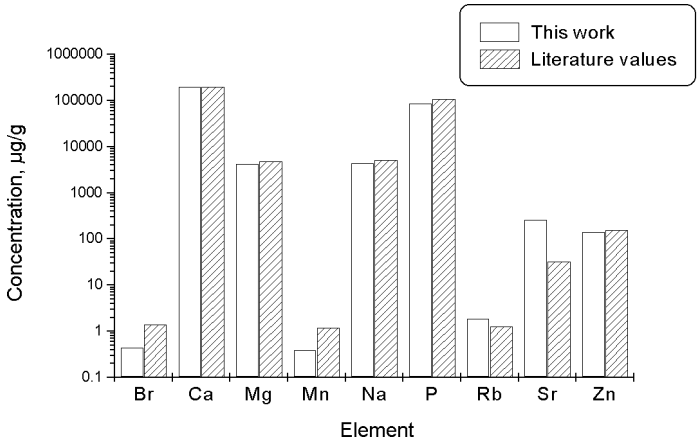

Fig. 1. Comparison of trace elements found in porcine cortical rib bones and literature values (wet weight basis)

These differences found for Zn and Ba deposition on porcine bones may be related to the different types of food consumed by these two animal species. Figure 1 shows a comparison of trace elements obtained for porcine cortical bone in this work with those presented by SAMULDRALWAR and ROBERTSON.[7] Concentrations obtained for Ca, Mg, Na, P and Zn are similar to literature values, however, our results presented higher concentrations for Rb and Sr and lower for Br and Mn.

In conclusion, our results confirmed that the INAA is a suitable method for bone analysis because of its multielemental character, the absence of a destruction step, and its good quality results. The reproducibility of the results obtained in the analysis of a sample of cortical and trabecular tissues indicated that the procedure defined for cortical and trabecular tissue treatment was appropriate to obtain homogenous samples. However, the calcination process was not suitable for whole the bone treatment due to the loss of Br and Cl.

Cortical and trabecular tissues presented different levels of element concentrations. This fact indicates that trace elements in samples of these tissues have to be studied separately.

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