APPLICATION OF NEUTRON ACTIVATION ANALYSIS AND INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY TO INVESTIGATE LANTHANUM AND LANTHANIDES CONTENT IN INTACT HUMAN RIB BONE

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Introduction

Lanthanum (La) and the lanthanides (Ln) are the elements, numbered 57-71 of the periodic table. La and Ln occur more frequently than their name implies. Contents of a number of lanthanides in the earth's crust is higher than, for example, copper. The rarest of the lanthanides – Tm, is much more common than silver. The most abundant La and Ln are cerium, lanthanum and praseodymium. Most often, La and Ln are found in crystallized form in fossils and shales.

In the second half of the 20 century begins the widespread use of La and Ln in the petroleum industry, nuclear power plants and metallurgy of special alloys. La and Ln have also been used as luminophores in the manufacture of color monitors and fluorescent lamps. Additionally, a large quantity of La and Ln gets into the environment during the production of phosphate fertilizers. Hence, the use of phosphate fertilizers significantly increased the content of La and Ln in the arable layer of soil.[1]

Lanthanum and the lanthanides get into the human body not only with food, drinking water and air, but also as therapeutic agents. La and Ln were used for many years for their anticoagulant and antiemetic properties in medicine. It is also proposed to use lanthanide containing compounds for therapeutic care of chronic renal failure patients, as well as a treatment of bone density disorders, such as osteoporosis[2-4]

There is increasing evidence that La and Ln administered ‘‘in vitro’’ or to experimental animals may influence a number of biological processes.[5-11] This effect is due to the similarity of chemical properties of lanthanide and alkaline earth metals. Chemical similarity allows ions of La and Ln to replace not only the ions of Ba, Ca, Mg, etc., but also transition metal ions in many macromolecular systems, including enzymes. At the same time, the replacement of La and Ln ions with the ions of alkaline earth elements is impossible. Lanthanum and the lanthanides also form stable compounds with phosphates.

According to studies conducted with the radionuclides La and Ln are bone-seeking elements. Bone is a tissue where the turnover these elements is extremely slow and its biological half-lives in bone were estimated to be from few years up to tens years. The long biological half-lives of lanthanides provide bone with several important features as a subject for environmental monitoring. First, element level in bone at any given time is an integral of intake (exposure) over the past several years to decades. Second, lanthanides accumulate in the bone more than in other tissues of human body. This means that even low-level exposure can be detected if the exposure has continued for a long time. Third, due to the large weight of skeleton, the element level in bone reflects its total body content. Thus, bone is suitable index medium for evaluation of low-level and long-term intake (exposure) and the body burdens of lanthanides.

Before bone analysis can be applied to monitoring environmental exposure it is necessary to establish normal values related to gender, age, inhabitancy and some other factors. As far as we know such data have not yet been obtained and/or published.

In the present study, La and Ln contents in human ribs were analyzed with three objectives in mind. The first objective was to use intact bones, the second one was to perform measurements on residents of a non-industrial region in the Central European part of Russia, and the third objective was to investigate age- and gender relations.

All studies were approved by the Forensic Medicine Department of Obninsk City Hospital and the Medical Radiological Research Center Ethical Committees.

Materials and methods

Samples of human rib bone were obtained at postmortems from intact cadavers (38 female and 42 male, 15 to 55 year old) within 24 h of death. Each death had resulted from automobile accidents, falls, shootings, stabbing, hanging, acute alcohol poisoning, or hypothermia. 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.

The majority of samples were taken from the 3rd, 4th, 5th, or 6th rib from the right side. A tool made of titanium and plastic was used to clean samples off soft tissues and blood. Samples were freeze dried until constant mass was obtained. A titanium scalpel was used to cut thin cross-sections of the rib weighing about 50-100 mg. Two sub-samples were received from each rib bone sample: one sub-sample for instrumental neutron activation analysis with using spectrometry of long lived radionuclides (INAA-LLR), and last one for inductively coupled plasma mass spectrometry (ICP-MS). 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. The sub-samples for ICP-MS were decomposed in autoclaves.

The contents Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Tm and Yb were measured by ICP-MS. Details of the samples decomposition, the spectrometer parameters, the main parameters of mass-spectrum measurements, the element content calculation, and the results of quality control were presented in our earlier publication concerning the ICP-MS of human bones.[12]

The contents Ce, Eu, Gd, La, Lu, Nd, Sm, Tb, and Yb were estimated by INAA-LLR too. Details of nuclear reactions, radionuclides, gamma-energies, methods of analysis and the results of quality control were presented in our earlier publication concerning the chemical elements of human bones.[13]

Two certified reference materials Tea Leaves INCT-TL-1 and Mixed Polish Herbs INCT-MPH-2 were analyzed simultaneously with rib samples to estimate the precision and accuracy of results. The samples of certified reference materials were treated in the same way as the rib samples.

Using standard programs, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, and percentiles with 0.025 and 0.975 levels were calculated for trace element contents. The reliability of difference in the results between the female and male cohorts as well as between two age groups was evaluated by Student's *t*- test.

Results

Table 1 depicts our data for Ce, Eu, La, Lu, Nd, Sm, Tb, and Tm mass fractions in samples of certified reference materials and the certified (or informative) values of these materials.

Table 2 represents our data for La and Ln mass fractions (mean± SEM, upper limit of the mean, detection limit) in intact human rib-bone received by INAA and ICP-MS.

The comparison of published data[13-16] with our results for chemical element contents in the rib bone of women and men is given in Table 3. Because a number of values for chemical element mass fractions were not expressed on a dry weight basis in the above works, we calculated these values using published data for water and ash contents in the rib bone.[17,18]

We used the entire dataset for both females and males (taken separately) seeking to detect the presence of gender-related differences (Table 4).

To estimate the effect of age on the investigated parameters, we examined two age groups: one comprised a younger group with ages ranging from 15 to 35 years and the other comprised older people with ages ranging from 36 to 55 years. The results for females and males, taken together, are shown in Table 5.

Table 1. ICP-MS data of La and Ln contents (M±SD) in Certified Reference Materials (mg/kg on dry weight basis)

Element	Tea Leaves (INCT-TL-1)		Mixed Polish Herbs INCT-MPH-2	
	Certificate	This work result	Certificate	This work result
Ce	0.790±0.076	0.791±0.027	1.12±0.10	1.26±0.31
Eu	0.050±0.009	0.046±0.002	0.0157±0.0018	0.0172±0.0028
La	1.00±0.07	0.974±0.012	0.571±0.046	0.616±0.147
Lu	0.017±0.002	0.018±0.001	0.0090±0.0015	0.0037±0.0002
Nd	0.81*	0.858±0.008	0.457±0.091	0.551±0.138
Sm	0.180±0.022	0.187±0.001	0.0944±0.0082	0.103±0.027
Tb	0.027±0.002	0.030±0.002	0.0135±0.0011	0.0115±0.0015
Tm	0.017*	0.016±0.001	-	0.0041±0.0001
Yb	0.120±0.013	0.113±0.005	0.0527±0.0066	0.0251±0.0002

* - non-certified values.

Table 2. Contents (M± SEM, upper limit of the mean, detection limit) of La and Ln in intact human rib bone investigated by INAA and ICP-MS (mg/kg, dry weight basis)

Element	INAA-LLR upper or detection limit	ICP-MS M±SEM
Ce	≤0.03	0.029±0.002
Dy	-	0.0020±0.0003
Er	-	0.0011±0.0002
Eu	≤0.001	<0.0007 DL
Gd	<0.25 DL	0.0015±0.0001
Ho	-	≤0.00053
La	<0.05 DL	0.020±0.002
Lu	<0.003 DL	≤0.00024
Nd	<0.1 DL	0.011±0.001
Pr	-	0.0032±0.0004
Sm	<0.01 DL	0.0014±0.0001
Tb	<0.03 DL	0.00041±0.00005
Tm	-	≤0.00006
Yb	<0.03 DL	0.00072±0.00007

M - arithmetic mean; SEM – standard error of mean, DL - detection limit.

Table 3. Median, min and max value of means of La and Ln contents in human rib bone according to data from the literature in comparison with our results (mg/kg on dry weight basis)

Element	Published data [13-16]			This work
	Median (n)	Minimum [Reference]	Maximum [Reference]	M±SEM
Ce	1.4 (2)	<0.03 [13]	2.7 [16]	0.029±0.002
Dy	≤0.2 (2)	<0.01 [15]	<0.4 [14]	0.0020±0.0003
Er	≤0.17 (2)	<0.01 [15]	<0.33 [14]	0.0011±0.0002
Eu	≤0.038 (4)	<0.001 [13]	<0.19 [14]	<0.0007 DL
Gd	≤0.25 (3)	<0.008 [15]	<0.47 [14]	0.0015±0.0001
Ho	≤0.047 (2)	<0.004 [15]	<0.09 [14]	≤0.00053
La	≤0.3 (4)	<0.05 [13]	0.86 [16]	0.020±0.002
Lu	≤0.004 (3)	<0.003 [13]	<0.09 [14]	≤0.00024
Nd	≤0.1 (3)	<0.02 [15]	<0.33 [14]	0.011±0.001
Pr	≤0.05 (2)	<0.01 [15]	<0.09 [14]	0.0032±0.0004
Sm	≤0.01 (3)	<0.004 [15]	<0.4 [14]	0.0014±0.0001
Tb	≤0.03 (3)	<0.004 [15]	<0.09 [14]	0.00041±0.00005
Tm	≤0.047 (2)	<0.004 [15]	<0.09 [14]	≤0.00006
Yb	≤0.03 (3)	<0.004 [15]	<0.33 [14]	0.00072±0.00007

n – number of all references; M - arithmetic mean; SEM – standard error of mean.

Table 4. Effect of gender on mean value (M±SEM) of La and Ln contents in the intact human rib bone (mg/kg on dry weight basis)

Element	Effect of gender		
	Females n=38	Males n=42	*p* t-test
Ce	0.0243±0.0032	0.0326±0.0036	N.S.
Dy	≤0.0012	0.0023±0.0003	-
Er	≤0.0065	0.0013±0.0003	-
Gd	0.0014±0.0002	0.0017±0.0002	N.S.
Ho	≤0.00023	≤0.00056	-
La	0.0162±0.0023	0.0228±0.0030	N.S.
Lu	≤0.00022	≤0.00026	-
Nd	0.0093±0.0013	0.0120±0.0016	N.S.
Pr	0.0026±0.0004	0.0038±0.0006	N.S.
Sm	0.0012±0.0002	0.0016±0.0002	N.S.
Tb	≤0.00029	0.00043±0.00005	-
Tm	≤0.00004	≤0.00008	-
Yb	≤0.00059	0.00087±0.00013	-

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

Table 5. Effect of age on mean value (M±SEM) of La and Ln contents in the intact human rib bone (mg/kg on dry weight basis)

Element	Effect of age		
	15-35 year n=36	36-55 year n=44	*p* t-test
Ce	0.0177±0.0021	0.0373±0.0036	≤0.001
Dy	0.0010±0.00003	0.0025±0.00003	≤0.001
Er	0.00053±0.00026	0.00138±0.00028	≤0.05
Gd	0.00090±0.00006	0.00203±0.00019	≤0.001
Ho	≤0.00051	≤0.00054	-
La	0.0124±0.0013	0.0266±0.0031	≤0.001
Lu	≤0.00024	≤0.00024	-
Nd	0.0064±0.0007	0.0141±0.0016	≤0.001
Pr	0.00170±0.00020	0.00441±0.00060	≤0.001
Sm	0.00072±0.00004	0.00198±0.00021	≤0.001
Tb	0.00025±0.00006	0.00049±0.00006	≤0.01
Tm	≤0.00004	≤0.00010	-
Yb	0.00056±0.00006	0.00081±0.00009	≤0.05

M - arithmetic mean, SEM – standard error of mean.

Fig. 1 shows the individual data for of La, Ce, Pr, Nd, Sm, and Gd in the human rib bone and lines of trend (exponential and linear) with age.

Using the exponential functions of La and Ln contents in the intact human rib bone (Fig. 1) the changes for 70 years of life were calculated (Table 6).

Table 6. Increasing of La and Ln contents (mg/kg on dry weight basis) in the intact human rib bone for 70 years according to the exponential functions (Fig. 1).

Element	Effect of age (exponential function)		
	Content (I) 1 year	Content (II) 71 year	(II) / (I)
Ce	0.00551	0.0810	14.7
Dy	0.000106	0.00749	70.5
Er	0.000032	0.00992	305
Gd	0.000311	0.00403	12.9
La	0.00424	0.0475	11.2
Nd	0.00208	0.0283	13.6
Pr	0.000522	0.0106	20.3
Sm	0.000208	0.00335	16.1
Tb	0.000103	0.000762	7.4
Yb	0.00021	0.00183	8.9
Σ	0.0133	0.195	14.6

Discussion

Detected means for the chemical element contents (mean ± SD) in the certified reference materials obtained in this work were in good agreement with the means of all certified values and within the 95% confidence intervals (Table 1). This indicates an acceptable accuracy of the results on La and Ln in intact rib bones samples.

The contents of Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb and Yb (10 elements) were measured by ICP-MS in all or a major portion of rib-bone samples (Table 2). The contents of Ho, Lu, and Tm (3 elements) were determined only in a few samples of collection. The upper limit of the mean values for the elements was found as the normalized sum of all individual contents and of the detection limits where the contents were not measured. The contents of Eu were lower than detection limits in all samples (<0.0007 mg/kg on dry weight basis). The upper limit of the mean values for Ce and Eu was estimated by INAA too. The contents of Gd, La, Lu, Nd, Sm, Tb, and Yb were lower than detection limits of INAA in all samples (mg/kg on dry weight basis): Gd<0.25, La<0.05, Lu<0.003, Nd<0.1, Sm<0.01, Tb<0.03, and Yb<0.03. Data received by two methods were in a full agreement.

The obtained means for Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb, and Yb as shown in Table 3, are one-two orders of magnitude lower than the median of mean value of previously reported data. The upper limit of means for Ho, Lu, and Tm is one-three orders of magnitude lower, than previously reported medians of upper limits. The detection limits of ICP-MS show that the contents of Eu in intact rib-bone of health men are at least one order of magnitude lower, than previously reported data.

No statistically significant gender-related differences were detected for all studied La and Ln (Table 4).

Comparison of mean values of the contents of La and Ln in the rib in two age groups (Table 5) revealed a statistically significant increase with the age of the content of Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb, and Yb. Thus, our data indicate the age-dependent accumulation of all La and Ln, when it was possible to determine the average content of elements and other statistical parameters. Age-dependent increase of the content of Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb, and Yb in the rib follows exponential and not liner trend (Fig.1). The exponential accumulation of La and Ln in the rib of healthy people living in a non-industrial, ecologically safe region indicates the presence of the global increase in the content of these elements in the environment.

If the accumulation of La and Ln in the rib occurs at an exponential rate, then during a period of over 70 years of lifespan (from the 1st year to 71 years) the content of Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb, and Yb would increased one-two orders of magnitude (Table 5). The total content of La and Ln in the bone tissue (Table 5) reaches the level of a few tenths of a mg per kg and becomes comparable with the content of minerals, such as B, Ba, Br, Cr, Cu, Mn, Mo, Ni, Pb, Rb, Ti, and one or two mathematical orders of magnitude higher than the content of Bi, Cd, Co, Hg, Sb, Sc, Se, Sn, U, V, Y, and Zr. [12]

Conclusions

In comparison with INAA, ICP-MS is a more appropriate analytical tool for the determination of La and Ln content in the bone tissue. Out of the total number of La and Ln, the current state of the ICP-MS allows to determine means for Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb and Yb (10 elements) and the upper limit of means for Ho, Lu, and Tm (3 elements).

For the first time, the average value of the content of Ce, Dy, Er, Gd, La, Nd, Pr, Sm, Tb and Yb, as well as the upper limit of means for Ho, Lu, and Tm were determined in the rib tissue of a healthy man.

There were no statistically significant differences found in the content of La and Ln in the rib bone of women and men.

Additionally, our data reveal that there is exponential accumulation of La and Ln in the bone tissue of healthy individuals that live in a non-industrial region. This indicates a global increase of the content of La and Ln in the environment.

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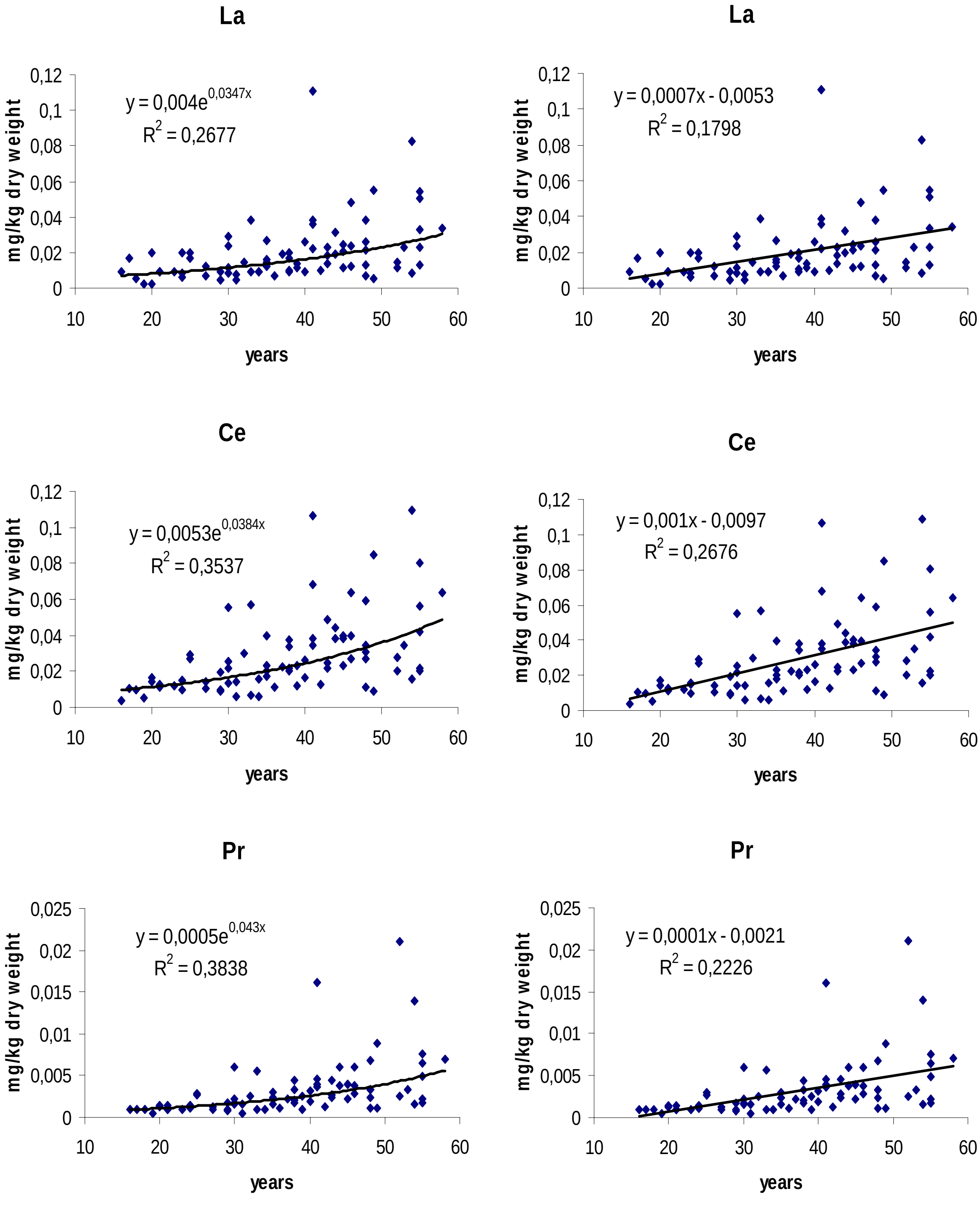

Fig. 1. Individual contents of La, Ce, Pr, Nd, Sm, and Gd in the human rib bone and lines of trend (exponential and linear) with age.

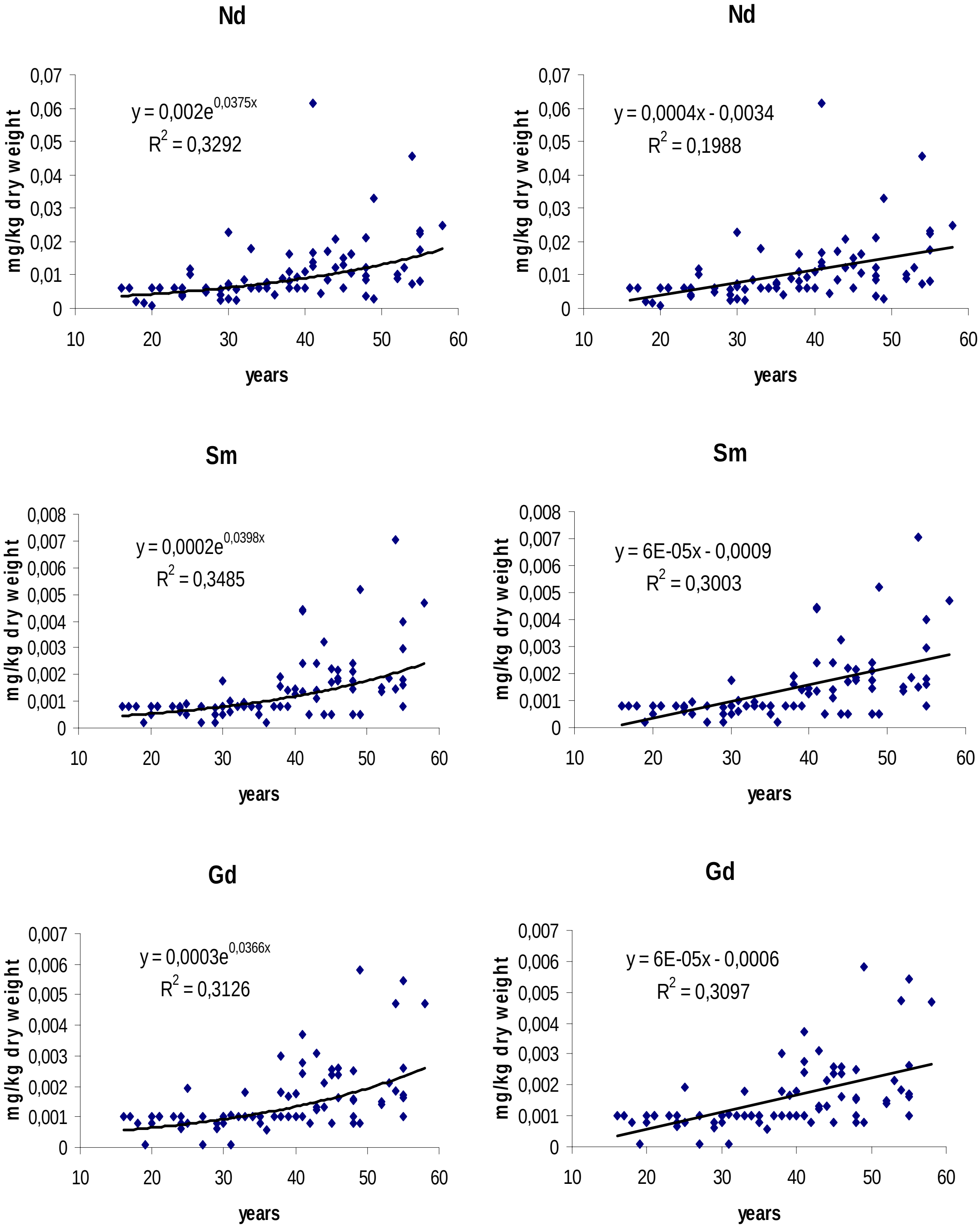

Fig. 1 (continuation). Individual contents of La, Ce, Pr, Nd, Sm, and Gd in the human rib bone and lines of trend (exponential and linear) with age.

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