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Ca and Mg determination from inhabitants of Brazil using neutron activation analysis

Cibele B. Zamboni · Laura C. Oliveira · Luciana Kovacs · Sabrina Metairon

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Abstract In this study the neutron activation analysis (NAA) technique was applied to determine Ca and Mg in whole blood from inhabitants of Brazil for the purpose of establishing concentration ranges indicative of sex and age. The initiative to perform these measurements is related to the increase in heart disease. According to recent statistics from WHO, the average is one death due to heart attack in Brazil, every five minutes. The measures were performed considering lifestyle factors (non-smokers, non-drinkers and no history of toxicological exposure) of Brazilian inhabitants. A healthy group constituted of male ($n = 94$) and female ($n = 84$) blood donors, ages between 18 and 70 years and above 50 kg, was selected from the blood banks and hematological laboratories of Brazil. The influence of sex was also investigated considering several age ranges (18–29, 30–40, 41–50, >50 years). The results show significant differences when a comparison is made by sex and age and may be useful to identify or prevent clinical diseases. These results emphasize the need to perform periodic evaluation of Ca and Mg in blood.

Keywords NAA · Blood · Calcium · Magnesium · Reference values · Heart disease

C. B. Zamboni · L. C. Oliveira · L. Kovacs · S. Metairon (✉)
Instituto de Pesquisas Energéticas e Nucleares – IPEN/CNEN-SP, Centro de Reatores de Pesquisa (CRPq), Cidade Universitária, Avenida Prof. Lineu Prestes, 2242, São Paulo, SP 05508-000, Brazil
e-mail: metairon@live.com

Introduction

Most of the calcium (Ca) and magnesium (Mg) in the body are stored in bone and only a tiny amount of these minerals (<1%) are present in the blood. Ca and Mg balance each other in the body and are two of the most important minerals that humans need to survive. Mg aids in the absorption of Ca in the body. This allows Ca to enter in the system faster, resulting in a quicker response to any bone or tissue ailments needing extra attention. The combination of the two elements working in harmony leads to an overall bone health and a number of other beneficial outcomes [1, 2]. The body needs these electrolytes to build and fix bones and teeth, for proper muscle function, for cardiovascular health, and other biological activities. In blood the calcium is important mainly for its role in clotting while the magnesium is needed to move other electrolytes, such as potassium and sodium, into and out of cells and also to help the body to process fat and protein. Most people who have low or high levels of Ca or Mg may not show any symptoms until the levels become greatly altered. However, serious clinical diseases can be avoided keeping these electrolytes in blood within the normal range [3].

Calcium is essential for many functions in the body. Most of it is used for building and maintaining strong bones and teeth, and healthy gums and is also important in the absorption of vitamin B. Ca in bones needs to be continually replaced, which is why proper levels of Ca are needed throughout life. In fact the need for this mineral increase with age, as the body becomes less efficient at absorbing calcium from food. Other benefits of Ca are: to reduce the risk of osteoporosis usually associated with low Ca and Mg levels, to reduce the levels of premenstrual syndrome or PMS, malabsorption syndrome (an alteration in the ability of the intestine to absorb nutrients adequately), as well as contraction of the muscles that make up the walls of small arteries [4]. While high levels of Ca in blood (hypercalcemia) [5] can frequently be associated with some endocrine diseases like thyrotoxicosis, Sarcoidosis, Addison's disease, and multiple endocrine neoplasia types I and IIA [6], it also can affect the pacemaker that controls the heart rate [7].

The crucial health benefits of Mg include solving or preventing thrombus formation, [8, 9] pathogenesis of coronary and cardiovascular diseases, as well as changes in serum lipids, which can increase the risk of atherosclerosis and that may also lead to anorexia, neuromuscular hyperactivity, which if untreated can contribute to cardiac arrhythmias or even heart attacks, strokes and other adverse effects [4]. An adequate intake of magnesium is essential to keep arteries relaxed, normal blood pressure and regular heart rate [10].

Statistics from the World Health Organization (WHO) [11] indicate an average of one death from myocardial infarction in Brazil occurs every five minutes. Althought men have a higher risk of heart attack than women (DATASUS) [12], this gap is minimized with advancing age. According to Andrade (2001) [13] the estrogen hormones are responsible for the protection of women before menopause and its decrease (postmenopausal) has been considered the main reason for development of cardiovascular disease among women. According to Viana et al. (2003) [14], in the 90s, the number of women in Brazil with heart disease has doubled in relation to estimates recorded in the 70s. Currently 20 to 30% of patients seeking cardiac care are postmenopausal women, ranging from 40 to 58 years. In addition, early menopause (before age 40 years) is associated with an increase of two to three times the risk of heart attack (Freitas 2006) [15].

In this study, Ca and Mg measurements in blood of Brazilian inhabitants were taken considering the influence of age and gender. These data can be useful in identifying and/or possibly preventing diseases of high incidence in Brazil such as osteoporosis [16] and cardiovascular disease [17].

This study is part of a larger project: Determination of reference values for element concentrations in human and animal-model fluids using NAA, currently in development at IPEN, in collaboration with blood banks, hematological laboratories of Brazil and several research centers [18–24]. Among of the objectives of this project is to increase awareness among donors, patients and the general public, of the need to carry out these clinical examinations as a routine procedure.

Experimental

The biological samples came from several blood banks in Brazil. Ethical approval for the study was obtained from the Ethics Committee authority. For this investigation a healthy group constituted by male ($n = 94$) and female ($n = 84$) blood donors, ages between 18 and 65 years old and above 50 kg, were selected. This selection involved inhabitants with no history of toxicological exposure, non-smokers, low alcohol consumption, including non-drinkers. The volunteers (male and female blood donors) were arranged in four groups by age (GI: 18–29, GII: 30–40, GIII: 41–50 and GIV: 51–68 years).

Volunteers were screened for Hepatitis B and C, AIDS, Syphilis and Chagas and positives for any one of these tests were excluded. For whole blood sample preparation, about 0.5 mL of whole blood was collected in a plastic vacuum tube (without anticoagulants) attached to the donor's arm. Immediately after the collection, before the blood coagulation, aliquots of 100 (± 0.5%) and 200 μL (± 0.5%) were transferred to Whatman filter paper ($\sim$2.5 cm^2), using a calibrated micropipette, and dried using an infrared lamp. For this investigation the whole blood samples were prepared in duplicate. Aliquots of standard solutions of Ca and Mg were also transferred to filter paper and prepared in the same manner as the biological samples. This procedure was applied due to some advantages: simultaneous evaluation of Ca and Mg using small quantities of whole blood (few μL) and the viability of the storage of the sample for a long period (which depends only the durability of paper filter) for future reexamination, without the need for any refrigeration.

The blood samples and standards were irradiated for 240 s in the pneumatic station in the nuclear reactor (IEA-R1, 2-4 MW, pool type) at IPEN. The thermal neutron flux utilized ranged from 6.5×10^{12} to 3.2×10^{13} cm^{-2} s^{-1}. After the irradiation, the activated materials and background radiation were gamma-counted for 900 s using a high resolution HPGe detector (FWHM = 1.89 keV) connected to an ADCAM multichannel analyzer (ORTEC 919E) and a PC computer. The concentrations of Ca and Mg were obtained using ^{49}Ca ($T_{1/2} \sim 9$ min, $E\gamma = 3{,}098$ keV) and ^{27}Mg ($T_{1/2} \sim 9$ min, $E\gamma = 1{,}012$ keV), respectively. Data reduction was done using in-house software. For analytical-quality control IAEA A-13 animal blood was used. The filter paper (blank) was also analyzed using the same irradiation conditions.

Results and discussion

For analytical-quality control IAEA A-13 animal blood was used and the results are presented in Table 1. The accuracy evaluation by Z-score test ($Z < |2|$) indicate the adequacy of the method for all elements determined. Some impurities such as Al and Cl were identified in the filter paper but they do not interfere.

Table 1 Element concentrations obtained in the analysis of AIEA A-13 reference material

Element mg kg^{-1}	Certified values	This work mean ± SD	RSD %	Er %	Z score
Br	22 ± 11%	24 ± 0.2	9.2	9.1	0.8
Ca	286 ± 19%	298 ± 32	10.7	4.2	0.2
Mg	99 ± 29%	118 ± 14	11.9	19.2	0.7
K	2500 ± 14%	2668 ± 320	12	7	0.5
Na	12600 ± 8%	12419 ± 269	2.2	1.5	1.8

RSD relative standard deviation, *Er* relative error

Table 2 Blood concentrations of Ca and Mg for inhabitants of Brazil by sex

Elements, gL^{-1}	All MV ± 1SD (range)[a] $n = 178$	Man MV ± 1SD (range)[a] $n = 94$	Women MV ± 1SD (range)[a] $n = 84$
Ca	0.290 ± 0.096	0.281 ± 0.104	0.302 ± 0.083
	(0.098–0.482)	(0.073–0.489)	0.136–0.468
Mg	0.035 ± 0.011	0.034 ± 0.011	0.036 ± 0. 011
	(0.013–0.057)	0.012–0.056	0.014–0.058

n number of samples analyzed in duplicate, *MV* mean value

[a] Confidence interval of 95% usually adopted for clinical practices

Table 3 Blood concentrations of Ca and Mg for inhabitants (Man) of Brazil by age (years)

Ca, gL^{-1}	18–29 $n = 26$	30–40 $n = 24$	41–50 $n = 28$	>50 $n = 16$
Range[a]	0.051–0.439	0.057–0.505	0.145–0.485	0.028–0.504
Mean	0.245	0.281	0.315	0.266
±1SD	0.097	0.112	0.085	0.119
Maximum	0.408	0.477	0.446	0.524
Minimum	0.124	0.092	0.182	0.094
Median	0.256	0.293	0.378	0.252
Mg, gL^{-1}	**18–29 $n = 26$**	**30–40 $n = 24$**	**41–50 $n = 28$**	**>50 $n = 16$**
Range[a]	0.008–0.056	0.018–0.046	0.014–0.062	0.016–0.060
Mean	0.032	0.032	0.038	0.038
±1SD	0.012	0.007	0.012	0.011
Maximum	0.059	0.046	0.054	0.054
Minimum	0.022	0.021	0.026	0.022
Median	0.027	0.031	0.052	0.041

n number of samples analyzed in duplicate

[a] Confidence interval of 95% usually adopted for clinical practices

Table 2 summarizes the results for Ca and Mg, respectively, as a function of sex. The average blood concentrations of Ca and Mg, grouped by age, are summarized in Table 3, for males, and Table 4 for females.

As seen in Table 2, there are no statistically significant differences ($p > 0.05$) for either Ca or Mg between males and females.

Table 3 reports the data of Ca and Mg in blood for males by age groups I through IV. For Ca, the mean is modestly increased in the 41–50 age group, but this increase is not statistically significant. However, when the ranges are used as reference (case of clinical practices), they are different suggesting the needs to consider the limits established by age. For Mg the mean values for all groups are compatible considering ± 1SD.

Table 4 shows the results of Ca and Mg in blood for females. For Ca, lower values were found in GI (18–29) and GIV (>51) age groups, but this decrease is not

Table 4 Blood concentrations of Ca and Mg for inhabitants (Woman) of Brazil by age (years)

Ca, gL^{-1}	18–29 $n = 32$	30–40 $n = 22$	41–50 $n = 14$	>50 $n = 16$
Range[a]	0.116–0.432	0.234–0.470	0.235–0.431	0.067–0.455
Mean	0.274	0.352	0.333	0.261
±1SD	0.079	0.059	0.049	0.097
Maximum	0.427	0.456	0.383	0.444
Minimum	0.168	0.249	0.252	0.136
Median	0.255	0.369	0.350	0.2395
Mg, gL^{-1}	18–29 $n = 32$	30–40 $n = 22$	41–50 $n = 14$	>50 $n = 16$
Range[a]	0.021–0.049	0.028–0.060	0.022–0.062	0.007–0.047
Mean	0.035	0.044	0.042	0.027
±1SD	0.007	0.008	0.010	0.010
Maximum	0.051	0.054	0.061	0.042
Minimum	0.026	0.033	0.032	0.009
Median	0.034	0.041	0.042	0.030

n number of samples analyzed in duplicate

[a] Confidence interval of 95% usually adopted for clinical practices

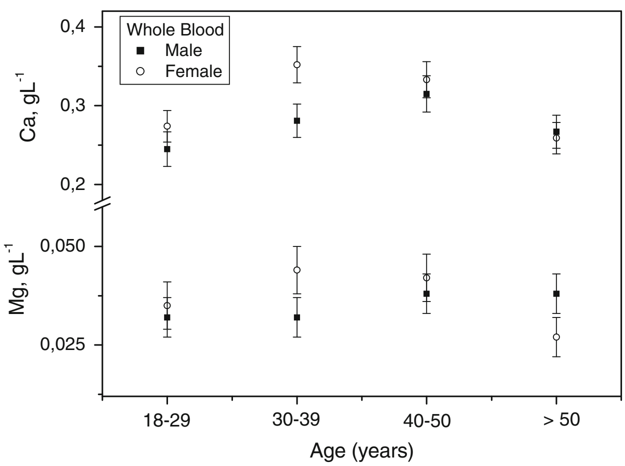

Fig. 1 Ca and Mg concentration in blood of inhabitants from Brazil as a function of sex and age

significant. When a comparison is performed between ranges for all age groups (Table 4), the lower limit for GI (0.116–0.432 gL^{-1}) and mainly GIV (0.067–0.455 gL^{-1}) are very different, suggesting that these intervals must be considered in clinical practices. For Mg (also Table 4) the mean values for all groups are overlap within ± 2SD.

A summary of the results for males and females is shown in Fig. 1 expressing a similar behavior for both sexes. Although many biological factors can be associated with the behaviour for Ca and Mg (Fig. 1), these results emphasized the need to use the intervals established for Ca and Mg in blood based on sex and age. Furthermore, for women it is also important to extend its evaluation to others subgroups, mainly for women in early menopause (before age 40) as well as for the older groups (>60 years) due to evidence that Mg deficiency in blood is increased with age and can cause metabolic changes that contribute to heart disease [15].

Conclusions

The indicative intervals for Ca and Mg in blood show non-significant differences when a comparison is performed by gender but, by age, the limits are different and must be considered for checking the clinical status of the subject.

The correlation of the low levels of Mg and Ca in blood with heart disease and osteoporosis, respectively, as well as the correlation of the high levels of Ca in blood with Addison's disease, emphasizes the needs to check routinely the Ca and Mg levels in blood in older groups (>50 years), mainly women.

We intend to improve the statistical data, mainly for Group IV, due to a wide age range investigated in this study (51–68 years old), dividing this group into two others (51–60 and 61–68 years old) for better follow-up of the decrease in element concentrations in the blood. Recently, a modification introduced in the Resolution—RDC no 153 – 14- June 2004, from National Health Surveillance Agency (ANVISA) [25], expanded the range for blood donation for youth aged 16 to 17 years old (with parental permission) and elderly (65–68 years old). Although this modification has not been regularly approved, this resolution will facilitate the collection of blood for investigation of Ca and Mg in these age groups.

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