

Determination of mineral constituents in duplicate portion diets of two university student groups by instrumental neutron activation

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Concentrations of 15 elements were determined simultaneously in duplicate portion diets of two university student groups from São Paulo University consisting of nine women (20–23 years) and ten men (20–24 years). The diet samples were prepared by either freeze-drying or drying in a ventilated oven. About 100–200 mg of diets were irradiated for 2 minutes and 8 hours in the IEA-R1m research reactor and Br, Ca, Cl, Co, Cr, Cs, K, Fe, Mn, Mg, Mo, Na, Rb, Se, and Zn were determined by instrumental neutron activation analysis (INAA). The average daily intakes found in the women and men groups were: 2.1 and 4.3 mg of Br, 501 and 707 mg of Ca; 3.1 and 6.0 g of Cl; 12 and 25 mg of Co; 15 and 36 µg of Cs; 53 and 63 µg of Cr; 5.1 and 10.8 mg of Fe; 1.3 and 2.8 g of K; 134 and 306 mg of Mg; 1.3 and 4.1 mg of Mn; 134 and 302 mg of Mo, 2.0 and 4.1 g of Na; 2.4 and 4.6 mg of Rb; 29 and 41 µg of Se; 6.2 and 10.6 mg of Zn, respectively. The daily intakes of Ca, Se and Zn in both groups and Fe in the women groups appeared to be below the U.S. RDA recommendations. For the elements Na and Cl the daily intakes were higher than the recommended values by RDA.

Introduction

In the recent years, there has been considerable interest in the determination of levels of trace elements in food, mainly because of their nutritional role and important functions in the human organism. Several biochemical and functional disturbances may occur when the human organism has micronutrient deficiency. There are various reports in the literature indicating the association of many diseases with mineral deficiencies, such as cancer, diabetes and the cardiovascular disease. Although the mechanisms responsible for these impairments are poorly understood, there is no doubt that micronutrients and trace elements are essential for human health.^{1,2} Consequently, studies that present adequate information about the diet and the nutritional status of subjects are important to relate diet to the healthy status.

Estimates of essential and other trace element intake through the daily diets of different populational groups are being carried out periodically by the Radiochemistry Division of IPEN/CNEN in collaboration with the Department of Food and Experimental Nutrition of the University of São Paulo.^{3–5} In these studies the diets were collected by the duplicate portion method, which is considered most appropriate when a small group is evaluated.

The aim of the present study is to estimate the micronutrients and trace element intakes in duplicate portion diets of two student groups by INAA. It is a part of a broader study designed to examine the nutrient intake and food habits of university students in São Paulo related with the nutritional status.

Experimental

Subjects

The study was carried out during the years 1998–1999 in two university student groups each consisting of nine women and ten men. All participants were studying at São Paulo University. The participants, aged 20 to 24, were healthy as shown by evaluation of their anthropometric characteristics (weight, height, body mass index – BMI, triceps skinfold-TSF, arm muscle circumference and body fatness measurements) and biochemical parameters.

Sampling

Pre-cleaned polyethylene containers were supplied to each student to collect the meals and beverages consumed during two days for females and three days for males. Twenty-four hour duplicates of all the meals were collected, in a total of 47 different diet samples. The diets were collected by the students themselves after a previous training.

Sample preparation and storage

The individual samples of each participant were prepared and analyzed separately. The inedible portions (like bone, peel of fruits) were discarded and the equivalent of the food consumed was weighed and stored in a refrigerator. Two different drying processes were carried out: (a) the female diets were dried in a ventilated oven at 60 °C until constant weight.

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Afterwards the diet samples were pulverized and homogenized in a stainless steel knife mill and kept at 4 °C before analysis; (b) the men diets were freeze-dried for about 48 hours and were homogenized in a domestic blender, which was coated with Teflon and equipped with titanium blades.

Instrumental neutron activation analysis

To determine the micronutrient and trace elements in the student diets by INAA, the samples were irradiated and counted in two regimes. The first irradiation for 2 minutes in a thermal neutron fluence rate of $10^{11} \text{ n}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ with the aid of pneumatic tube facility of the IEA-R1m reactor was used, for the determination of short and some intermediate half-life radioisotopes. The second irradiation for 8 hours in a thermal neutron fluence rate of $10^{13} \text{ n}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ was employed to determine long-lived radioisotopes. Primary elemental standards and biological reference materials were simultaneously irradiated with the samples for standardization and quality control purposes, respectively.

After the 2-minute irradiations, two countings were made: the first after a 2-minute decay in which the elements Ca, Mg and Cl were determined and the second counting after a 90-minute decay for the determination of the elements K, Mn and Na. After the 8-hour irradiations, the intermediate half-lived Br and Mo and the long-life radioisotopes of the elements Cr, Co, Cs, Fe, Se, Rb and Zn were determined after 5 to 7 and 15 to 20-day decay times, respectively.

All diet samples, the primary standards and reference materials were measured using a counting system with an Ortec EG&G high resolution solid state Ge detector (POP TOP Model 20190) with a resolution of 1.9 keV for the 1332 keV γ -ray peak of ^{60}Co . This detector was coupled to an EG&G Ortec ACE8K card and associated electronics. Spectrum analysis was performed using the VISPECT2 software.

Results and discussion

The validation of the analytical method applied in this work was carried out by analysis of biological reference materials SRM 1566a Oyster Tissue and SRM 1577b Bovine Liver. The results obtained showed good agreement with the certified values (Table 1).

The daily intakes of the micronutrient and trace elements were calculated on the dry weight basis. The daily intake values were calculated by multiplying the concentrations of the elements in the individual 24-hour diet samples by the total weight of food consumed by each person. The average dry weight of daily intake of 254 g/day for women and of 411 g/day for men were determined. Table 2 shows the mean and standard deviation and the range of concentration values for each microelement and trace elements determined by INAA. The results of average daily intakes of Br, Ca, Cl, Co, Cr, Cs, Fe, K, Mg, Mn, Mo, Na, Rb, Se and Zn are shown in Table 3. The daily intakes for all elements assayed were higher in males than in females. The highest differences were observed for the elements Mn, Cs, Mg, Mo, K and Fe.

Table 1. Micronutrient and trace elements in certified reference materials (dry weight) by INAA

Element, unit	NIST SRM 1566a Oyster Tissue		NIST SRM 1577b Bovine Liver	
	This work (Mean \pm SD)*	NIST Value**	This work (Mean \pm SD)*	NIST Value**
Br, mg/kg	56 \pm 5	(55)	8.9 \pm 0.9	(9.7)
Ca, g/kg	2080 \pm 165	1960 \pm 190	126 \pm 18	116 \pm 4
Cl, g/kg	7492 \pm 333	8290 \pm 140	2844 \pm 187	2780 \pm 60
Co, ng/kg	0.65 \pm 0.06	0.57 \pm 0.11	0.26 \pm 0.03	(0.25)
Cr, ng/kg	1.37 \pm 0.16	1.43 \pm 0.46	0.28 \pm 0.05	–
Cs, ng/kg	0.033 \pm 0.006	(0.02)	0.012 \pm 0.003	–
Fe, mg/kg	494 \pm 36	539 \pm 15	167 \pm 9	184 \pm 15
K, g/kg	7853 \pm 922	7900 \pm 470	10246 \pm 606	9940 \pm 20
Mg, g/kg	1090 \pm 60	1180 \pm 170	656 \pm 110	601 \pm 28
Mn, mg/kg	11.8 \pm 0.9	12.3 \pm 1.5	10 \pm 1	10.5 \pm 1.7
Mo, mg/kg	1.07 \pm 0.07	–	3.39 \pm 0.27	3.5 \pm 0.3
Na, g/kg	3887 \pm 244	4170 \pm 130	2452 \pm 75	2420 \pm 60
Rb, mg/kg	2.9 \pm 0.3	(3)	12.0 \pm 1.0	13.7 \pm 1.1
Se, mg/kg	2.01 \pm 0.23	2.21 \pm 0.24	0.61 \pm 0.07	0.73 \pm 0.06
Zn, mg/kg	785 \pm 48	830 \pm 57	110 \pm 7	127 \pm 16

* Mean and standard deviation of 4 individual determinations.

**Certified values are those associated with uncertainties, non certified values are in parentheses.

Table 2. Essential elements in student diets determined by INAA

Element, unit	Female group		Male group	
	Mean \pm SD	Range	Mean \pm SD	Range
Br, mg/kg	8.3 \pm 2.6	5.6 – 14.5	9.9 \pm 4.4	3.6 – 27.3
Ca, g/kg	2256 \pm 1234	468 – 5663	1569 \pm 671	483 – 3337
Cl, g/kg	12664 \pm 3292	5981 – 18831	13712 \pm 3955	7020 – 24192
Co, μ g/kg	60 \pm 22	22 – 128	52.8 \pm 26.6	10.8 – 117
Cr, μ g/kg	244 \pm 109	83 – 460	150 \pm 137	28 – 712
Cs, μ g/kg	92 \pm 49	23 – 232	74 \pm 41	25 – 176
Fe, mg/kg	22 \pm 7	14 – 47	24 \pm 6	13 – 39
K, g/kg	5388 \pm 1179	3526 – 8310	6128 \pm 1458	3473 – 9717
Mg, g/kg	568 \pm 98	377 – 828	653 \pm 161	363 – 1072
Mn, mg/kg	5.2 \pm 1.3	3.1 – 8.0	9.1 \pm 5.1	4.0 – 26.2
Mo, μ g/kg	542 \pm 148	321 – 1053	691 \pm 231	312 – 1170
Na, g/kg	8430 \pm 2355	3871 – 12767	9446 \pm 3086	4696 – 18833
Rb, mg/kg	10.4 \pm 3.4	4.3 – 20.5	10.0 \pm 3.9	4.8 – 21.5
Se, μ g/kg	138 \pm 68	52 – 297	99 \pm 37	43 – 180
Zn, mg/kg	22 \pm 6	11 – 34	23 \pm 8	9.7 – 42

Table 3. Average daily intake of micronutrients and trace elements by the student diets

Element, unit	Female group			Male group		
	Mean \pm SD	Range	RDA	Mean \pm SD	Range	RDA
Br, mg/d	2.1 \pm 1.8	0.9 – 4.9	–	4.3 \pm 2.6	1.1 – 14.5	–
Ca, mg/d	501 \pm 282	110 – 1074	1200 ^a	707 \pm 414	151 – 1815	1200 ^a
Cl, mg/d	3065 \pm 1342	918 – 5114	750 ^c	5972 \pm 2567	1831 – 11878	750 ^c
Co, μ g/d	12 \pm 8	3.7 – 34	–	25 \pm 20	3.1 – 105	–
Cr, μ g/d	53 \pm 29	19 – 113	50 – 200 ^b	63 \pm 46	5.3 – 207	50 – 200 ^b
Cs, μ g/d	15 \pm 13	3.1 – 49	–	36 \pm 30	6.5 – 112	–
Fe, mg/d	5.1 \pm 2.9	1.6 – 12.4	15 ^a	10.8 \pm 4.9	3.5 – 21	10 ^a
K, mg/d	1311 \pm 604	479 – 2642	2000 ^c	2781 \pm 1323	597 – 5366	2000 ^c
Mg, mg/d	134 \pm 51	48 – 217	280 ^a	306 \pm 135	93 – 607	350 ^a
Mn, mg/d	1.3 \pm 0.3	0.3 – 2.1	2.0 – 5.0 ^b	4.1 \pm 2.8	1.2 – 12.1	2.0 – 5.0 ^b
Mo, mg/d	134 \pm 48	51 – 250	75 – 250 ^b	302 \pm 135	107 – 652	75 – 250 ^b
Na, mg/d	2032 \pm 891	569 – 3397	500 ^c	4070 \pm 1782	1375 – 9247	500 ^c
Rb, mg/d	2.4 \pm 1.0	0.7 – 4.8	–	4.6 \pm 2.8	0.9 – 10.9	–
Se, μ g/d	29 \pm 10	17 – 52	55 ^a	41 \pm 19	21 – 111	70 ^a
Zn, mg/d	6.2 \pm 2.2	1.4 – 9.2	12 ^a	10.6 \pm 5.9	2.6 – 24	15 ^a

^a Recommended dietary allowance for females and males (19–24 years).

^b Estimated safe and adequate daily dietary intakes.

^c Minimum requirements of healthy person.

Minimum dietary allowances of sodium, potassium and chloride (e.g., 500, 2000 and 750 mg/day, respectively, in adults) have been recommended.⁶ Actual intakes of sodium and chloride are considerably greater than those suggested in many populations. The US National Research Council⁶ recommends that daily intakes of sodium chloride be limited to 6 g (2.4 g of sodium) or less. In the male diets this value was exceeded in 85% of the subjects studied. Reduction of salt intake is, therefore, indicated since excessive sodium intake is considered to be a risk factor in hypertension and osteoporosis.⁷ On the other hand, considering the possible beneficial effect of potassium in hypertension, the potassium intake over the minimum requirement has been recommended.⁸

The food intakes of the female group forming the basis of this duplicate portion study were relatively small in most cases, so the essential trace intakes were far below the recommended levels.

The average daily intake of calcium was below the RDA value of 1200 mg/day in both groups, it was adequate in 42% of females and 59% of males (Fig. 1). These small values are due to the low consumption of milk and dairy products.

As to the other essential trace elements determined, like magnesium, selenium, zinc, the daily intakes were below the RDA values in both groups, only iron in the male diets reached the RDA recommendations.

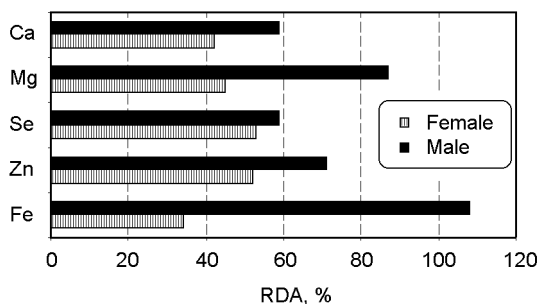


Fig. 1. Adequacy of essential trace element intake in student diets

The low value of iron obtained in the female diets is worrying, since in about 88% of the female diets analyzed the daily intakes of Fe were under the 2/3 RDA value, which is considered to be of concern, according to RDA.

Conclusions

Instrumental neutron activation analysis was applied to determine 15 elements in duplicate portion diets in two different student groups. Due to the small food intake of the female students, this group shows deficiency for the essential elements investigated, mainly for iron, which can bring serious consequences to these student's health status.

The sodium and chloride intakes in the male group were considerably higher than the minimum requirement.

Due to the fact that the soil of the region of São Paulo, where the study was made, is poor in selenium, as already shown in previous studies,^{3,5} the Se intakes were below, indicating a possible need for supplementation of the diets with this element.

Also for zinc, which is a component of more than 200 metalloenzymes, and is important for many biological functions, there seems to be also a need for diet supplementation, since the intakes evaluated in this study are very low compared to the recommended values.

The results obtained show that, there is a tendency to a low intake of essential trace elements studied in both groups studied, which is more pronounced in the female student group. It can be pointed out that a lower consumption of food compared to the male group could be responsible for the low ingestion of essential elements.

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References

1. World Health Organization (WHO), Trace Elements in Human Nutrition and Health, Geneva, 1996.
2. E. O. UTHUS, C. D. SEABORN, *J. Nutr.*, 126 (1996) 2452S.
3. D. I. T. FÁVARO, M. L. T. HUI, S. M. F. COZZOLINO, V. A. MAIHARA, M. J. A. ARMELIN, M. B. A. VASCONCELLOS, L. K. YUYAMA, G. T. BOAVENTURA, V. A. TRAMONTE, *J. Trace Elements Med. Biol.*, 11 (1997) 129.
4. V. A. MAIHARA, M. B. A. VASCONCELLOS, M. B. CORDEIRO, S. M. F. COZZOLINO, *Food. Add. Contam.*, 15 (1998) No. 7, 782.
5. D. I. T. FÁVARO, V. A. MAIHARA, D. MAFRA, S. A. SOUZA, M. B. A. VASCONCELLOS, M. B. C. CORDEIRO, S. M. F. COZZOLINO, *J. Radioanal. Nucl. Chem.*, 244 (2000) 241.
6. National Research Council, Recommended Dietary Allowances, 10th ed., National Academy of Sciences, Washington, D.C., 1989.
7. S. MIZUSHIMA, K. TSUCHIDA, Y. YAMORI, *Clin. Exp. Pharm. Phys.*, 26 (1999) No. 7, 573.
8. A. FLYNN, *Adv. Food Nutr.*, 36 (1992) 209.