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# Application of NAA to the determination of mineral and trace elements in Brazilian diets at IPEN/CNEN/SP

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In the present work, the concentration of the 14 elements Br, Ca, Cl, Co, Cs, Fe, K, Mn, Na, Rb, Sc Se and Zn have been determined by INAA in diets of four different groups: (a) 19 pre-school children, (b) 18 healthy adults, (c) 23 elderly people living in private institutions and (d) 19 patients with chronic renal failure (CRF). The contents of proteins, lipids and carbohydrates were also analysed in the diets. The daily intakes of the elements analysed were compared to the recommended values set by RDA or WHO.

## Introduction

Inorganic elements are essential components of diets and their deficiency or excess may result in serious disorders. The effects of trace element deficiency are most severe during development and growth and they are especially important for infants and children, but are also of great importance for the elderly and in conditions of illness.

The nutrients are necessary during all stages of life and especially during the illness states. Abnormalities in trace mineral metabolism have been observed in some kinds of diseases. In cases of chronic renal failure for instance, several studies have shown that an abnormal zinc metabolism can be observed in patients.[1–3] The mechanisms responsible for these changes have not been fully established and the contribution of trace element toxicity or deficiency to the symptoms of renal disease is uncertain.[4] Patients with renal disease who follow special diets restricted in protein (like conservative treatment) are at additional risk of developing zinc or iron deficiency, because foods rich in trace elements such as meat and marine fish are restricted in these diets.

Recently studies have added to the knowledge of the role of trace elements in human health.[5] Diet is the single most important source of all elements for human beings in a natural environment. Hence it is important to know the composition of the diets (major, minor and trace elements). Analysis of actual diets have been undertaken in many countries since the 80's and are still being carried out, in order to verify the intake data for essential and toxic elements.

In the last few years in Brazil, neutron activation analysis has been used for this purpose in several studies[6–8] at the Radiochemistry Division of the Instituto de Pesquisas Energéticas e Nucleares, IPEN-CNEN/SP, because of its high precision, accuracy and sensitivity.

In the present work, diets from 4 different groups such as children, healthy adults, elderly, and patients with chronic renal failure were studied. The purpose of this work was to assess mineral and trace elements content of these diets by using neutron activation analysis techniques. Also, the results obtained for proximate composition are presented and discussed.

## Experimental

### *Pre-school children, adults and elderly groups*

A group of 19 pre-school children whose mean age was 5.1 years (range 3.4–6.9 years), weight 19 (13.7–29.7) kg, and height 110 (94.5–125) cm was chosen. These children remained in the nursery the whole day, eating two complete meals and snacks.

The elderly group (23 total) was selected from private institutions of the urban region of São Paulo. Their mean age was about 84 (range 70–95) years, weight 55 (33–91) kg, and height 157 (135–172) cm.

The healthy adults group (9 males and 9 females) was selected among the teachers and employees of the São Paulo University. Their mean age was about 43.8 years.

In these three groups, duplicate diets, representative of normal meals, were collected. Details of the sample preparation have been given previously,[8] so only a summary will be given here.

The duplicate portions were put in pre-cleaned polyethylene containers, weighed and refrigerated. The children's diets were mixed and homogenized in a domestic blender that was coated with teflon and equipped with titanium blades. After this, the samples were freeze-dried and again homogenized in the blender.

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*Table 1.* Characteristics of patients with CRF group

| Data | Uremics patients (median and range) | Healthy adult group (median) |
|---|---|---|
| Age females, years (*n*) | 45.1 (9) | 43.7 (9) |
| Age males, years (*n*) | 52.1 (10) | 43.8 (9) |
| Creatinine clearance, ml/min | 29.5 (25.2–43.3) | Not determined |
| Serum urea, mg/dl | 78.0 (72.7–111) | Not determined |
| Serum creatinine, mg/dl | 2.6 (2.2–3.8) | Not determined |
| Hemoglobin males, g/dl | 12.5* | 14.4* |
| Hemoglobin females, g/dl | 11.1* | 13.0* |
| Hematocrit males, % | 38.5* | 45.4* |
| Hematocrit females, % | 37.0* | 41.0* |
| BMI, kg/m$^2$ | 23.8 | 24.0 |

* $p<0.05$.
*n*–Number of individuals.

The elderly diets were placed in stainless steel trays and dried in a ventilated oven. Afterwards the samples were pulverized and homogenized in a knife mill. The diets of the healthy adults were collected by the previously trained participants, and after this the diets were prepared for analysis in the same way as for the elderly group.

*Patients with chronic renal failure*

The protocol for the study with these patients was approved by the São Paulo Hospital (UNIFESP) Research Committee. After giving their informed consent, nineteen nondialyzed patients with Chronic Renal Failure (CRF) (10 males and 9 females) were included in this study. In Table 1 the characteristics of these patients are shown. The hematocrit and hemoglobin values of the patients obtained were different from the adult values at level of $p<0.05$. All volunteers were apparently healthy and the dietary prescription for them was to consume 0.6 g protein/kg/day and 35 kcal/kg/day.

Stature and weight were used in the Body Mass Index (BMI) (weight/stature$^2$ in kg/m$^2$) to describe levels of body composition in adults group. Each patient completed 3-day food records. Before the collection of food records, each patient underwent a standardized session on how to keep food records, including instructions on how to estimate the portion sizes. After this, all patients' meals were prepared in the laboratory from the food records and dried in an oven at 60 °C for 24 hours. Then, the ground foods were analysed for proximate composition.[9]

*Dietary assessment*

Total fat and protein contents were determined using the Soxhlet extraction and Micro-Kjeldahl methods, respectively. Ash contents of the samples were determined after dry ashing at 550 °C for 10 hours. The carbohydrate amount was obtained as the difference between total dry weight and the sum of protein, fat and ash content. The conversion factors of 4 kcal/g of protein and carbohydrate and 9 kcal/g of fat were used to calculate the energy value (Association of Official Analytical Chemists, 1990).[9]

*Instrumental neutron activation analysis*

Instrumental neutron activation analysis was used in this work to determine quantitatively the minor and trace elements in diet samples. Individual or multielemental solutions were used as primary element standards. The synthetic standard preparation was described in a previous paper.[7] The reference materials Oyster Tissue (NIST SRM 1566a), Total Diet (NIST SRM 1548), and Citrus Leaves (NIST SRM 1572) were used for checking the precision and accuracy of the method.[7]

Aliquots of about 200 mg of diet samples and reference material (weighed in pre-cleaned polyethylene bags) were placed, with the synthetic standard, into polyethylene or aluminium containers, and irradiated in the IEA-R1 research reactor. Short and long irradiations were carried out depending on the half-life of the induced radionuclides.

*Table 2.* Composition and adequacy of children, healthy adult, elderly and patient group diet samples

| Components of diets | Children diets | | Healthy adult group diet | | Elderly diet | | | Patient groups diet | |
|---|---|---|---|---|---|---|---|---|---|
| | Daily intake[a] | RDA[b] | Daily intake | RDA | Daily intake | RDA | | RDA | Recommendation to patients' diet |
| | | | | | | M | W | | |
| Energy, kcal | 866[c] | 1800 | 1841 | 2500 | 851 | 2300 | 1900 | 1150 | 35 kcal/kg/day |
| Protein, g | 32.7 | 24 | 80.7 | 56.5 | 28 | 63 | 50 | 38.7 | 0.6 g/kg/day |
| Fat, g | 28.1 | – | 54.8 | 83.3 | 19.8 | – | – | 25.8 | 30% |
| Carbohydrate | 120.7 | – | 268 | 375 | 139.3 | – | – | 218.7 | The rest of kcal |
| Fibre, g/day | – | – | – | 20–25 | 10.8 | 12 | 12 | – | 20–25 |
| Humidity, % | 5.22 | – | 6.3 | – | 4.78 | – | – | 7.7 | – |
| Ash, % | 3.42 | – | 3.8 | – | 3.00 | – | – | 3.09 | – |

[a] For a daily intake of 790 g, 753.3 g, 1150 g and 950 g for children, elderly, control and patients diets, respectively.
[b] RDA values: children from 4 to 6 years (RDA, 1989),[11] values for man (M) and for woman (W).
[c] Energy value calculated considering the carbohydrates with fibre fraction for children diets.

*Table 3.* Results for diet samples analyzed by INAA (dry weight)

| Element | Children diet | | Adult diet | | Elderly diet | | Patient group diet | |
|---|---|---|---|---|---|---|---|---|
| | Average±SD | Range | Average±SD | Range | Average±SD | Range | Average±SD | Range |
| Br, μg/g | 9.2 ± 3.0 | 5.5–16.6 | 7.5 ± 1.8 | 4.7–11.4 | 8.1 ± 1.6 | 3.9–12.2 | 6.2 ± 1.9 | 2.4–9.6 |
| Ca, mg/g | 2.2 ± 0.7 | 1.3–4.0 | 1.4 ± 0.8 | 0.5–3.6 | 1.6 ± 0.3 | 1.0–2.4 | 1.2 ± 0.5 | 0.5–2.4 |
| Cl, mg/g | 13.5 ± 5.0 | 4.6–27.5 | n.d. | – | 9.0 ± 1.1 | 7.4–11.0 | n.d. | – |
| Co, ng/g | 50.8 ± 15.8 | 23.2–84.1 | 79 ± 69 | 31–313 | 50.4 ± 14.3 | 27.6–79.1 | 49.4 ± 26 | 15–92 |
| Cs, ng/g | 85 ± 25 | 59–154 | 84 ± 37 | 27–165 | 71 ± 20 | 53–131 | 91 ± 72 | 28–340 |
| Fe, μg/g | 27 ± 4 | 22–37 | 41 ± 17 | 19–72 | 24.3 ± 5.9 | 16.4–39.7 | 26.3 ± 10.3 | 8.4–45 |
| K, mg/g | 7.1 ± 1.3 | 5.1–9.3 | 7.8 ± 4.1 | 4.3–17.9 | 5.2 ± 1.0 | 3.0–7.2 | 5.8 ± 1.8 | 3.3–10.6 |
| Mn, μg/g | 7.3 ± 2.6 | 4.6–14.8 | n.d. | – | 5.2 ± 2.2 | 3.1–10.7 | n.d. | – |
| Na, mg/g | 7.6 ± 1.7 | 5.0–10.6 | 8.0 ± 3.5 | 4.6–19.1 | 5.6 ± 0.6 | 4.7–6.8 | 6.5 ± 2.0 | 2.5–9.7 |
| Rb, μg/g | 11.9 ± 2.7 | 7.5–18.6 | 10.4 ± 3.6 | 5.1–17.7 | 9.1 ± 2.2 | 4.7–12.6 | 10.4 ± 2.6 | 4.3–15 |
| Se, ng/g | 133 ± 32 | 88–223 | 119 ± 48 | 68–227 | 138 ± 27 | 67–174 | 90 ± 34 | 38–155 |
| Sc, ng/g | 1.9 ± 0.9 | 1.1–5.0 | 1.6 ± 0.6 | 0.9–3.1 | 1.8 ± 0.4 | 1.0–2.6 | 1.3 ± 0.5 | 0.4–2.5 |
| Zn, μg/g | 24.5 ± 4.5 | 18.6–33.3 | 25.5 ± 7.2 | 12.9–38 | 16.3 ± 2.4 | 11.0–22.4 | 20.5 ± 6.7 | 7.3–38 |

n.d. – Not determined.

For determination of the short lived radioisotopes $^{38}Cl$, $^{27}Mg$, $^{56}Mn$, $^{24}Na$ and $^{42}K$, the samples and standard were irradiated for 2 minutes with a thermal neutron fluence rate of $10^{11}$ n·cm$^{-2}$·s$^{-1}$. For long irradiations, the samples and standards were irradiated for 8 hours at thermal neutron fluence rates of $10^{12}$–$10^{13}$ n·cm$^{-2}$·s$^{-1}$, and after different cooling times, the following radioisotopes were determined: $^{82}Br$, $^{47}Ca$, $^{60}Co$, $^{59}Fe$, $^{86}Rb$, $^{75}Se$, $^{46}Sc$ and $^{65}Zn$.

The gamma-ray spectra were obtained by using a counting system with an Ortec EG&G high resolution solid state Ge detector (POP TOP Model 20190) with 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

Table 2 presents the results of the proximate composition of diet samples analysed. All diets were low in energy, high in proteins for children and adults. In the elderly diets there was a protein deficit of 44% and 56% for males and females, respectively. Most of the patients showed energy intakes lower than 20 kcal/kg/day. There was marked reduction in caloric intake in patients and they were ingesting more than the recommendation of 0.6 g protein/kg/day (Table 2). These dietary intakes can accelerate the disease progression, where the uremic signs are highly dependent on protein intake.[10]

*Table 4.* Daily intakes of the essential elements analysed in the diet samples and recommendations (WHO,[5] RDA[11])

| Element | Children diet | | Adult diet | | Elderly diet | | Patient group diet | | WHO | RDA |
|---|---|---|---|---|---|---|---|---|---|---|
| | Average±SD | Range | Average±SD | Range | Average±SD | Range | Average±SD | Range | | |
| Br, mg/d | 1.8 ± 0.6 | 1.0–3.7 | 3.3 ± 1.2 | 1.2–5.8 | 1.7 ± 0.5 | 0.8–2.5 | 1.9 ± 0.9 | 0.9–4.3 | 1[b] | – |
| Ca, mg/d | 438 ± 143 | 241–777 | 636 ± 316 | 105–1275 | 377 ± 94 | 171–479 | 353 ± 163 | 134–674 | 400–500 | 800[c]–1200 |
| Cl, mg/d | 2725 ± 1261 | 971–6258 | n.d. | – | 1922 ± 494 | 814–2889 | n.d. | – | – | 750[d] |
| Co, µg/d | 10.2 ± 4.1 | 4.1–19.6 | 40 ± 45 | 7.3–177 | 10.9 ± 4.7 | 5.2–24.1 | 15.5 ± 9.7 | 3.6–38.3 | 2 | 3[e] |
| Cs, µg/d | 16.6 ± 4.4 | 10.6–25.6 | 36.3 ± 14.5 | 9.7–57 | 14.9 ± 4.9 | 8.9–26.2 | 27.8 ± 26 | 8.3–131 | – | – |
| Fe, mg/d | 5.3 ± 1.0 | 4.2–7.5 | 19 ± 11 | 6.6–49 | 5.2 ± 1.6 | 1.9–8.7 | 8.4 ± 4.4 | 3.1–18.2 | 10 (M),20 (F) | 10[f]; 15[h] |
| K, mg/d | 1408 ± 340 | 946–2176 | 3446 ± 1878 | 800–8881 | 1081 ± 256 | 582–1447 | 1845 ± 886 | 819–3980 | – | 2000[d] |
| Mn, mg/d | 1.5 ± 0.5 | 0.8–2.6 | n.d. | – | 1.1 ± 0.5 | 0.4–2.6 | n.d. | – | 2–3 | 2.0–5.0 |
| Na, mg/d | 1502 ± 408 | 872–2407 | 3681 ± 2320 | 1252–10800 | 1195 ± 278 | 525–1807 | 2031 ± 1050 | 934–4267 | – | 500[d] |
| Rb, mg/d | 2.4 ± 0.5 | 1.6–3.1 | 4.7 ± 2.0 | 1.3–7.0 | 1.9 ± 0.5 | 0.8–3.0 | 3.3 ± 1.3 | 1.2–5.8 | – | – |
| Se, µg/d | 26 ± 7 | 16–40 | 53 ± 27 | 15–129 | 30 ± 11 | 11–57 | 29 ± 15 | 12–71 | 70 | 70[g]; 55[h]; 20[i] |
| Sc, ng/d | 385 ± 211 | 206–1131 | 699 ± 278 | 332–1313 | 390 ± 140 | 110–690 | 445 ± 262 | 51–1184 | – | – |
| Zn, mg/d | 4.8 ± 1.1 | 3.3–7.4 | 11.6 ± 5.1 | 3.1–20.1 | 3.5 ± 1.1 | 1.5–5.9 | 6.6 ± 2.9 | 2.7–11.5 | 10–15 | 15[g]; 12[h]; 10[i] |

[a] Recommended dietary allowance or estimated safe and adequate daily dietary intake.
[b] Br (WHO) = 1 mg bromide ion per kilogram body weight per day.[5]
[c] Minimum requirements for older age groups and children at ages 1 to 10 years (RDA).[11]
[d] Minimum requirements of healthy persons (RDA).[11]
[e] Recommended daily dietary allowances in terms of vitamin $B_{12}$.
[f] RDA values for males, for elderly women and men and for children (RDA).[11]
[g] RDA values for males.[11]
[h] RDA values for female.[11]
[i] RDA values for children.[11]

Table 3 shows the mean and standard deviation and the range of values for each essential and trace element analysed by INAA. Daily intakes of elements were obtained by multiplying the concentrations in the individual diet samples by the total weight of food consumed by each person. The average amount of food consumed daily was about 800 g for children and elderly groups, and 950 g for patient groups, and 1150 g for adult groups. The mean daily intakes (in μg per person) of each element were obtained by adding together the daily intake calculated for each individual and dividing by the number of participants of the study (19 children, 18 healthy adults, 23 elderly, and 19 patients). The average daily intakes of essential and trace elements are presented in Table 4.

From Table 4 it can be observed that the diets of children and the elderly show deficiency for the essential elements Ca, Fe and Zn when their daily intake are compared with RDAs[11] or recommended values from WHO.[5] For selenium, only the diets of children attained RDA levels of dietary intake. As to the other elements, the dietary intake was in the ranges recommended as adequate and safe.

Ca, Fe and Zn deficiencies in children can impair their growth and development. Studies have also shown that the cognitive development and the immune system can be impaired by Zn and Fe deficiency.[12,13] The Fe deficiency also is considered a problem of public health in Brazil, because of low dietary intake and low bioavailability. Anemia is common in children populations.[14]

For the elderly, the deficiency of these elements can be very important especially for Ca, because of the high levels of osteoporosis in this population group. According to MILLER and WEAVER[15] the recommendation for this nutrient varies from 1200 to 1500 mg/day. On the other hand, Zn deficiency can be responsible for the impaired immune systems often found in people in this age group. Studies are being carried out in by our group in Brazil to verify if the zinc supplementation could improve their health status. Se is also important, because glutatione peroxidase enzyme needs Se for the antioxidant system of the body, which protects the cells from lipid oxidation.[5]

The patients' trace element intakes were reduced, showing deficiency for Zn, Se, Fe, and Ca. The low intake of these elements could be related to diet restrictions and could impair the general health status of these patients. These deficiencies can be related to many abnormalities in renal disease, such as lipid peroxidation, hipogeusia, impaired immune system, sexual impotence, anaemia, or osteodistrophy, since the elements Zn and Se are components of the antioxidant enzymes.

## Conclusions

Neutron activation analysis can be successfully used for the determination of some essential and trace elements in diets and consequently can supply information about the nutritional assessment of population groups.

From the results obtained in the present work we make the following conclusions.

(1) Child, adult and elderly diets have a low energy intake, due to low food consumption rates, although the percent distribution of the proteins, carbohydrates and lipids offered to them was within the limits set for Brazilian diets. These groups also showed deficiency for the essential elements Ca, Fe, and Zn when their daily intake are compared with RDAs[11] or WHO[5] recommended values. High Na intake was observed for all diets analysed in the present work.

(2) The patients group diet had low energy and high protein intake when compared to that recommended for people that suffer this kind of pathology. The low intake of Ca, Fe, Se and Zn could be related to diet restrictions and could impair the general health status of these patients.

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