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DETERMINATION OF SELENIUM BALANCE IN HEALTHY CHILDREN BY AAS-HYDRIDE GENERATION AND BY INAA TECHNIQUE

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Total daily Se intake was determined by duplicate diet collection, venous blood samples were taken and urine was collected over 24 h in order to measure selenium input and output in healthy, American and Hungarian children aged 8 to 17 living in Budapest. The American children consumed not only locally processed food. Food samples were weighed, mixed, homogenised and the Se content was determined by Instrumental neutron activation analysis (INAA). The Se concentration of blood, plasma and urine samples was determined by atomic absorption spectrometry-hydride generation (AAS-HG) after wet digestion.

Se intake calculated for wet weight was 62±18.5 μg/day in American children. In the Hungarian children the mean Se intake was about 35% less than in the Americans. Se concentrations in plasma were 0.84±0.16, in whole blood 1.13±0.17 μmol l^{-1} in the Americans, higher than those in healthy Hungarian children (0.64±0.10 and 0.83±0.12 μmol l^{-1}, respectively) of similar age and gender. Urinary Se output calculated for creatinin was higher in the children from abroad (27.0±9.5 μg Se/day/g creatinin) compared to Hungarians (11.0±5.0 μg Se/day/g creatinin).

Keywords: selenium balance, Instrumental neutron activation analysis (INAA), atomic absorption spectrometry-hydride generation (AAS-HG).

Selenium is an essential trace element, and its antioxidant role as a constituent of a selenoprotein in the enzyme glutathione peroxidase is well established (COMBS & COMBS, 1984). Dietary Se deficiency causes health problems in animals and humans (MOLNÁR et al., 1998, ŠOBAJIĆ-AKSENTIJEVIĆ et al., 1998). Se status in animals tends to reflect general levels of Se in soil (LEVANDER, 1987). Earlier studies found selenium levels in wheat grains and other grain crops from main agricultural regions of Hungary to be low in comparison to other European countries (GONDI et al., 1992). Some basic carbohydrate nutrients also indicated to low Se supply in Hungary (SZIKLAI-LÁSZLÓ et

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al., 2000). The aim of this study was to determine Se intake and output by two different methods in healthy, American and Hungarian children living in Budapest.

Selenium (Se) status depends mainly on Se intake in humans, which is determined by the Se content of the diet provided by the complete food chain. Se intake varies widely from region to region depending on the Se content of soil and its availability to plants. Hungarian soils have been proven to be low in Se, with concentrations being between 0.1–0.42 mg Se kg^{-1} soil.

Blood Se status – based on Se parameters of erythrocytes, whole blood and plasma as well as glutathione peroxidase activities of erythrocytes and plasma in healthy Hungarian children and adults – was low in comparison to several other, healthy, European populations (CSER et al., 1996).

The principal route of non-retained and excreted Se is the urinary output, which accounts for 50–60% of the intake in a well supplied population. The daily urinary Se excretion is associated with the plasma Se concentration and the dietary Se intake in healthy subjects with sufficient Se supply. Thus the contribution of various food groups to the overall dietary intake of selenium can differ markedly from one country to another and general dietary patterns could be very different. There are no balance studies on Se metabolism in children living in a geographical area with low Se supply (CSER et al., 2000).

The primary aim of this study was to optimise the selenium determination in biological and in food samples by the AAS-HG technique and to compare the results with the values obtained by the INAA technique. Se intake, blood status and urinary Se excretion of healthy Hungarian and American children living in Budapest, having different eating patterns and consuming local or largely mixed diet were compared with the help of these analytical methods.

1. Material and methods

1.1. Analysis of biological samples by atomic absorption spectroscopy (AAS)-hydride generation (HG)

The covalent hydride is formed by the addition of a basic sodium borohydride solution to an acidified sample that contains Se. The formed Se-hydride is passed into a quartz tube, which is heated by an air/acetylene flame where the hydride is broken up and the atomised Se is measured in the usual way by atomic absorption spectroscopy (TINGGI, 1999).

Measurements were carried out using UNICAM 929 AA spectrometer with UNICAM VP 90 vapour system. In order to measure with good sensitivity and reproducibility the measuring parameters were carefully optimised as detailed above. Instrumental conditions are summarised in Table 1.

Table 1. AAS-HG instrumental conditions

Wavelength	196.0 nm
Bandpass	Full 1.0 nm
Lamp current	75%
Background correction	D2
Purge gas type, flow	Argon, 280 ml min^{-1}
Fuel flow	0.8 l min^{-1}
Stabilisation time	30 s
Baseline delay	30 s
Measurement time	10 s

Table 2. INAA measuring parameters

Thermal neutron flux at the irradiation position	8.1×10^{13} n $cm^{-2}.s^{-1}$
Irradiation time	24 to 48 h
Cooling time	40 to 90 days
Measuring time	5, 15 and 20 h

1.2. Analysis of food samples by instrumental neutron activation analysis (INAA) and gamma-spectroscopy

Irradiations were performed in the WWR-M type 10 MW nuclear research reactor of the KFKI Atomic Energy Research Institute (Budapest, Hungary) (SZIKLAI-LÁSZLÓ et al., 2000). The parameters are summarised in Table 2.

Measurement of the γ-rays of ^{75}Se ($t_{1/2}$: 119.76 d; eγ: 136.0, 264.65 keV) was performed by the IBM PC/AT-486 based spectrometer equipped with a KFKI PCA-4KN type MCA card. The detector was CANBERRA Ge(Li) (with energy resolution of 1.82 keV and efficiency of 13.6% for the 1332.5 keV ^{60}Co line).

Gamma spectra evaluations and calculation of element concentrations were possible by the HYPERMET-PC program – an interactive version of the HYPERMET – for automatic peak evaluation, energy calibration and isotope identification, and the INAACNC program for concentration computation.

1.3. Sample digestion for AAS-HG measurements

Four ml of urine sample or 0.1 ml of blood sample was measured into Pyrex glass digestion tubes and was digested in conventional heating steps with 10 ml acid mixture (the acid mixture was prepared as follows: 750 ml cc. nitric acid +375 ml cc. perchloric acid +115 ml cc. sulphuric acid +260 ml bidistilled water). The digestion was completed when approximately 0.5 ml of colourless solution remained. After cooling 4 ml of 6 N hydrochloric acid was added and heated to 100 °C for 30 min to reduce Se(VI) to Se(IV). After the samples cooled down, they were transferred into calibrated tubes and diluted to 20 ml with bidistilled water.

1.4. Sampling and sample preparation for INAA measurements

The food samples collected over 24 h were homogenised. Aliquots of the samples were lyophilised and dried for 48 h at 80 °C to constant weight. One hundred to 300 mg samples were weighed and irradiated in high purity SUPRASIL quartz ampoules. Aliquots of selenium standard solution (Merck) were irradiated together with the samples.

2. Results and discussion

2.1. Optimisation of the AAS-HG method

To get the lowest possible detection limit, we determined the best conditions of the flow rates of gases such as acetylene and argon. The flow rate of acetylene determines the temperature of the flame. It was found that the highest signal could be achieved when the flow rate of acetylene was the lowest acceptable for the instrumentation. The best results were obtained when the flow rate was 0.8 ml min^{-1}.

In the case of argon as carrier gas with a low flow rate, the signal was very stable, but too low. When the flow rate was higher, the signal became higher, but the reproducibility became worse and the noise higher, probably because the gas stream whirled along the liquid drops (Fig. 1). The optimal flow rate was 280 ml min^{-1}.

The next step was to determine the best concentration of the sodium-borohydrate solution. According to our results, in the range of 1–2.5 g l^{-1} the height of the signal increased rapidly. When the concentration was higher than 3.5 g l^{-1}, the height of the peaks was just the same, but the reproducibility decreased, while noise increased again.

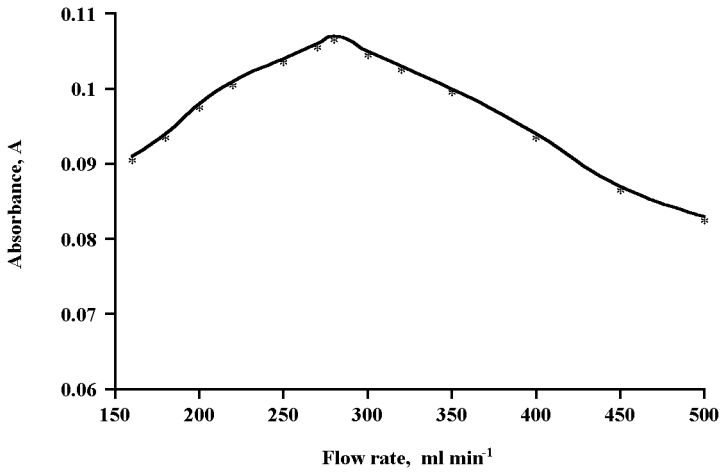

Fig. 1. Effect of argon flow rate on Se measurement (AAS-HG)

The optimal concentration was found at 3 g l^{-1} sodium-borohydrate (Fig. 2). The solution was prepared daily.

The concentrations of hydrochloric acid and sodium-hydroxide were studied at the same time because these parameters are not independent of each other (Fig. 3). The best results could be obtained when the HCl concentration was 2 N and the NaOH concentration was 0.8 g l^{-1}.

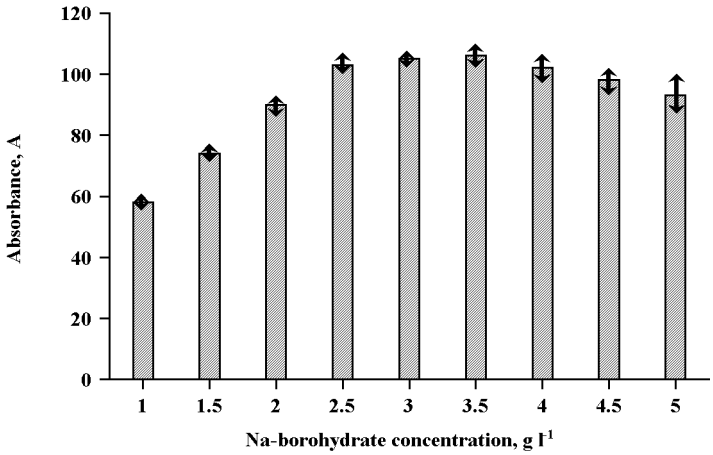

Fig. 2. Effect of Na-borohydrate concentration on Se measurement (AAS-HG)

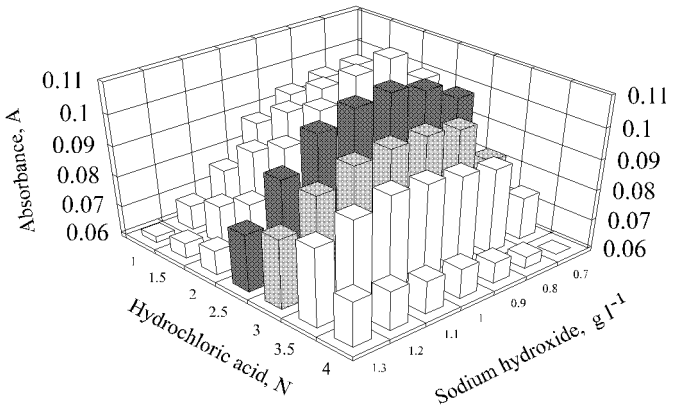

Fig. 3. Effect of acid and alkali concentration on Se measurements (AAS-HG, 10 ng l^{-1} Se standard)

Table 3. Quality control for AAS-HG and for INAA

	Certified levels (μg Se kg^{-1})	Measured values by AAS-HG (μg Se kg^{-1})	Measured values by INAA (μg Se kg^{-1})
NIST SRM 1567a Wheat Flour	1100 ± 200	1024 ± 149 (n=15)	1195 ± 78 (n=16)
NBS SRM 1568 Rice flour	400 ± 10	369 ± 21 (n=5)	
NIST SRM 1548 Total diet	245 ± 5	228 ± 8 (n=5)	240 ± 14 (n=5)
NBS SRM 1549 Non-fat Milk powder	110 ± 10	104 ± 12 (n=9)	105 ± 4 (n=5)

2.2. Quality control for AAS-HG and for INAA in reference materials

The analytical methods were compared by measuring different certified standard samples by both methods simultaneously. The results are summarised in Table 3. The values obtained by the two different methods were in good correlation and agreed to the certified data.

2.3. Clinical parameters of the children

Fifteen Hungarian and ten American children were selected, and they recorded their food intakes. Over two consecutive week days and a day of the weekend, they collected food duplicates and weighed them. Urine was collected in trace element free plastic containers. Blood samples were taken on the last day. The clinical parameters of children and the results of their diet are summarised in Table 4.

Table 4. Clinical parameters of children and results of their diet

	Americans	Hungarians
Numbers	10	15
Age median (years)	13.4	13.1
Age range (years)	8–17.5	8–18
Body weight (percentile)	25–97	25–97
Diet collection	duplicate	recorded, calculated
Protein intake g kg^{-1} b.w. M±SD	1.8±0.42	0.72±0.32
Carbohydrate intake g kg^{-1} b.w. M±SD	8.3±0.4	5.8±0.9
Fat intake g kg^{-1} b.w. M±SD	1.5±0.6	2.9±0.3
Calory intake kcal day^{-1} M±SD	2320±175	2280±128

Total daily Se intake was higher in Americans, 62 versus 41 μg/day. Caloric intake was similar in both groups. Americans consumed more protein and carbohydrates, while Hungarians had higher fat intake. Se concentrations of the plasma and the whole blood were higher in Americans (Fig. 4b). Urinary Se excretion was remarkably less in Hungarians compared to Americans (Fig. 4a). Se output as percentage of intake was 43.5% in the Americans and only 27% in the Hungarians (Fig. 4c), indicating a Se retention effect.

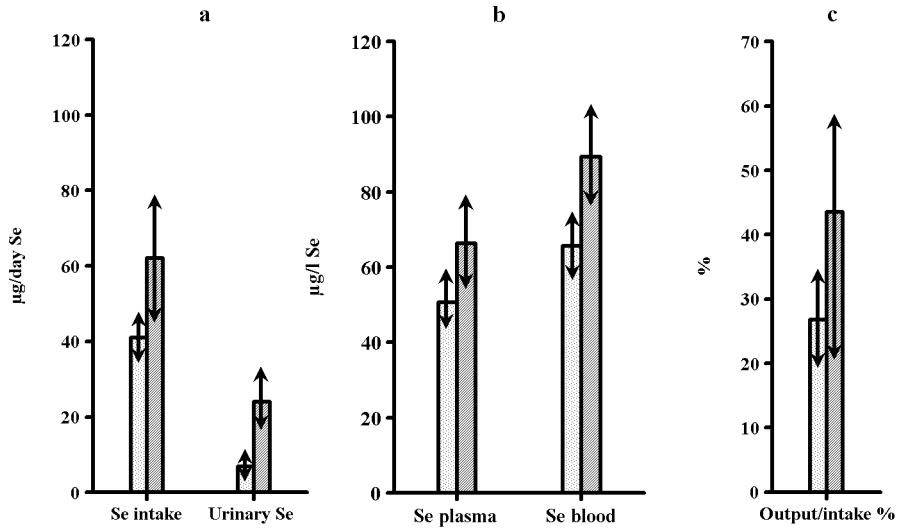

Fig. 4. Balance studies. a. Se intake and urinary output; b. Se in blood and in plasma; c. Se output/input. □: Hungarians; ■: Americans

3. Conclusion

When measuring the same standard materials, correlation between the results obtained by AAS-HG and INAA methods for Se determination were well comparable.

Se balance studies revealed that they are not suitable to define requirements, since humans maintain Se balance over a broad range of intakes (MERTZ, 1987). In spite of this statement the comparison of the balance results of the two healthy groups of children may give some information about habitual metabolic patterns.

We have come to the conclusion that locally produced food alone does not give enough Se supply. Foreign children consumed more cereals of foreign origin, more brown bread, milk and meat resulting in a higher Se level. The selenium concentration in whole blood and plasma was higher in the American children. Urinary selenium output was less in the Hungarian children who had lower Se intake and blood status than the others. These investigations could be more informative when they would be completed by the analysis of the Se forms and bonds via species measurements.

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