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### Trace elements in the blood of institutionalized elderly in the Czech Republic

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#### ABSTRACT

Whole blood selenium, copper, zinc and manganese concentrations of 197 institutionalized senior citizens (46 males, 151 females) aged 61–100 years (mean age 83.6 years) were determined using atomic absorption spectrometry and inductively coupled plasma mass spectrometry from two localities in the Czech Republic (Prague and Teplice) from 2009 through 2011. Geometric means (GM) of whole blood selenium (B-Se), copper (B-Cu), zinc (B-Zn) and manganese (B-Mn) levels were 74.0  $\mu$ g/l, 941.0  $\mu$ g/l, 5898.0  $\mu$ g/l and 10.9  $\mu$ g/l, respectively. Gender-related differences were found with significantly higher B-Cu levels in women and significantly higher B-Zn levels in men. The percentage of seniors with B-Se levels lower than 56  $\mu$ g/l (which is equivalent to the critical value of 45  $\mu$ g/l of Se in serum (S-Se)) was 8.6%. A negative correlation between age and B-Se levels was found. B-Zn positively correlated with serum albumin and prealbumin whereas a negative correlation between age and B-Zn was observed. Seniors with diabetes mellitus and atherosclerosis had higher B-Cu and B-Mn levels, respectively, than those without these diagnoses. The elderly from Prague had significantly higher B-Mn concentrations than those from Teplice. We found no evidence of serious essential elements deficiencies or excesses in the seniors who participated in this study.

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#### 1. Introduction

The nutritional status of seniors is a basic determinant of overall health. In the Czech Republic, the over 65 age group is rapidly increasing (Rambousková, Křížová, Dlouhý, Potočková, & Anděl, 2010) and health promotion programs for the elderly are becoming very important (Křížová, Brzyski, Strumpel, Billings, & Lang, 2010). The elderly are one of the most vulnerable population groups. Reduced nutrient and energy intake can quickly lead to undernutrition (Mowe, Bohmer, & Kindt, 1994), a condition that is becoming increasingly prevalent in the elderly population. By virtue of being elderly, this population is also faced with a variety of age-related diseases and chemical imbalances (i.e. some elements can accumulate in the body (Vahter, Åkesson, Lidén, Ceccatelli, & Berglund, 2007) (e. g. copper) or decrease (e. g. zinc) (Fox, Mukhopadhyay, & Ehrenwald, 1995; Wood, Suter, & Russell, 1995) during aging). The trace elements selenium, copper, and zinc have specific concentrations essential for human health. Most

trace elements are integral parts of metabolically important proteins, including enzymes (Malmström & Leckner, 1998; Rink & Gabriel, 2000). For example, selenium acts through a wide range of selenoproteins with an array of functions (Brown & Arthur, 2001; McKenzie, Arthur, & Beckett, 2002). Copper is involved in oxidereduction processes (Jacob & Milne, 1993; Johnson, Fischer, & Kays, 1992); zinc plays an important role in the immune system (Artacho, Ruíz-López, Gámez, Puerta, & López, 1997) and belongs to a group of essential trace elements required for the activity of several enzymes involved in DNA synthesis and macronutrients metabolism. It is known that the elderly are at risk of zinc deficiency (Briefel et al., 2000). The trace element manganese plays a role in mammalian metabolism and is associated with good health; however, its exact metabolic pathways are not yet entirely clear (Reilly, 2004). Manganese belongs to a group of trace elements with equivocal meaning relative to essential and adverse effects, primarily with regard to concentration-related neurotoxic effects (Mergler & Baldwin, 1997). Results presented here are part of a larger project entitled The Nutritional Status Assessment of Senior Citizens in Institutionalized Care. The central theme of the whole project was to assess the nutritional status of the institutionalized elderly population. One component of the

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assessment of nutritional status of the elderly included an analysis of essential elements in whole blood from a selected group of seniors. The results of this component analysis are presented here.

#### 2. Materials and methods

#### 2.1. Subjects

The study population was recruited from 9 institutions of social care (retirement homes and nursing homes) in the capital city of the Czech Republic (Prague) and from 2 institutions in a middlesized town in Northern Bohemia (Teplice) from 2009 to 2011. The study was approved by the Ethics Committee of the Third Faculty of Medicine, Charles University in Prague. All subjects in the study were informed about the planned examination and gave written informed consent. From 1069 randomly recruited subjects aged 61 yrs and over, blood samples for essential metal analysis (selenium, copper, zinc and manganese) were obtained from every fifth senior in the study. The analysis of essential elements was completed for 197 institutionalized seniors. In addition to the essential elements, biochemical parameters (albumin, prealbumin, transferrin, urea and creatinine) were also analyzed (publication of these results is in progress). Additionally, selected toxic metals (mercury, cadmium and lead) were also determined (data not shown).

For each subject in the study, information on age, gender, marital status, education, employment, last job, food consumption, food supplement use, type of diet, smoking habits, length of institutionalization, pharmacotherapy and anamnesis data regarding overall health were recorded. Information related to medical conditions and medications was obtained from personal medical histories. Some of the most common health issues of residents were ischemic heart disease (63.3%), hypertension (67.4%), diabetes mellitus (30.5%), hypercholesterolemia (9.2%), osteoporosis (17.6%) and Parkinson's disease (6.6%).

Blood samples were taken using a standard protocol. Blood was collected into trace metal-free tubes to avoid contamination. S-Sarsted Monovette® tubes containing heparin as the anticoagulant and appropriate siliconized needles were used. Specimens were frozen at  $-18\,^{\circ}\text{C}$  until analysis.

#### 2.2. Analyses

#### 2.2.1. Sample preparation

Blood samples were mineralized with a mixture of concentrated nitric acid and hydrogen peroxide in a microwave oven (MEGA 1200; Milestone) equipped with a FAM 40 evaporation rotor (to reduce external contamination). After mineralization, the resulting sample solutions were evaporated to a volume of approximately 0.1 ml and then diluted with demineralized water at the final volume of 5 ml – solution (A).

#### 2.2.2. Determination of selenium, copper and manganese

Prior to analysis, mineralized solutions (A) were diluted two and a half times using 1% (v/v) nitric acid. Measurements of Se, Cu and Mn were carried out using Inductively Coupled Plasma mass spectrometry (ICP-MS) on a Perkin Elmer quadrupole ICP-MS Elan DRC-e (Perkin ElmerSCIEX Instrument, Canada). An internal standard (a mixture of Ge, In and Re, final concentrations of standards in samples 10  $\mu g/l$ ) was added to all samples and standard solutions. Isotopes and internal standards chosen for determinations of individual elements and their limits of detection (LOD) and quantification (LOQ) are given in Table 1.

#### 2.2.3. Determination of zinc

Mineralized solutions of samples (A) were measured directly using Flame Atomic Absorption spectroscopy (FAAS) on a

**Table 1**Isotopes and internal standards chosen for the determination of individual elements using ICP MS, their limits of detection and quantification.

Element	Measured isotopes	Internal standard	LOD (µg/l)	LOQ (µg/l)
Selenium Copper Manganese	<sup>82</sup> Se <sup>+</sup> <sup>65</sup> Cu <sup>+</sup> <sup>55</sup> Mn <sup>+</sup>	In Ge Ge	0.87 0.61 0.04	2.91 2.05 0.13

LOD: limit of detection; LOQ: limit of quantification.

Perkin-Elmer AAS 3300 spectrometer at 213.9 nm. The LOD for zinc was 100  $\mu g/l$ .

#### 2.2.4. QA/QC

The accuracy of the measurement was checked using a certified reference material of whole blood Seronorm<sup>TM</sup> Trace Elements Whole Blood L-1 and L-2 (SERO, Norway). All determined values of elements under study were in good agreement with declared values.

#### 2.3. Statistical analyses

Data are shown as means  $\pm$  SD if they were found to be normally distributed after testing with the Kolmogorov–Smirnov test. The results of blood trace elements values were normalized after log-transformation so they are expressed in  $\mu$ g/l and presented as GM, 95% CI, minimal and maximal values and as percentages. The two-sample Wilcoxon-test was used. Other tests for statistical analysis included the Chi-square test of independency in contingency tables and Fischer's exact test to compare groups using contingency tables. Inter-group differences, linear regression analysis and Spearman correlations were used and p-values of less than 0.05 were accepted as statistically significant. All statistical analyses were performed using SPSS software for Windows, version 19.0.

#### 3. Results

The main population characteristics of the seniors involved in this study are presented in Table 2. Altogether 76.6% of the monitored group were women (mean age 84.5 yrs, age range 61-100 yrs) and 23.4% were men (mean age 80.8 yrs, age range 64-95 yrs). Women had a higher mean age than men and nonsmokers had a higher mean age than smokers (84.5 yrs vs. 84.2 yrs and 80.8 yrs vs. 73.3 yrs, respectively). Additionally, women from Prague had a higher mean age compared to women from Teplice. 94.4% of the monitored population were non-smokers, with only one person not providing information regarding smoking habits. Whole blood selenium, copper, zinc and manganese levels in the studied seniors are shown in Table 3. For comparison of our results with other studies, data from the literature, if expressed in µmol/l, were recalculated to µg/l. The GM value of B-Se and range was 74.0  $\mu$ g/l (36.3–209.7  $\mu$ g/l); seniors from Prague had non-significantly higher levels (74.5 µg/l) compared to those from Teplice (72.8 µg/l). There was no significant difference between men and women (74.8  $\mu$ g/l vs. 73.8  $\mu$ g/l) nor between smokers and nonsmokers (75.3  $\mu$ g/l vs. 74.1  $\mu$ g/l). A negative correlation between age and B-Se levels (r = -0.339; p < 0.000) was found (Fig. 1). Use of dietary supplements significantly increased B-Se levels in the elderly (78.0  $\mu$ g/l vs. 72.4  $\mu$ g/l; p < 0.05). However, the exact composition of the nutritional supplements taken by seniors was unknown. The proportion of subjects with B-Se concentrations lower than the critical value associated with higher risk of cardiovascular diseases (56  $\mu$ g/l) was 8.6% (N = 17). When comparing B-Se levels with diseases recorded on the health questionnaire, seniors with ischemic heart disease had statistically

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