

Original Research

Blood Selenium Concentration of Residents of Upper Silesia: Relation to Age and Gender

B. Kłapcińska^{1*}, S. Poprzęcki¹, A. Danch²

¹Department of Physiological and Medical Sciences, Academy of Physical Education,
ul. Mikołowska 72A, 40-065 Katowice, Poland

²Department of Experimental and Clinical Biochemistry, Medical University of Silesia, ul. Medyków 14, 40-752 Katowice, Poland

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Abstract

The aim of this study was to evaluate age- and gender-dependent differences in the selenium status of apparently healthy human subjects living in Upper Silesia. A total of 1380 individuals (925 male and 455 female) were examined for whole blood selenium. The subjects were subdivided into six groups according to age, the youngest included adolescents aged 10-15 years, and the oldest were centenarians. The mean Se content was relatively low (63.5 ± 18.1 µg/L), and it was significantly ($p < 0.01$) higher in men (66.3 ± 17.0 µg/L) than in women (58.1 ± 18.9 µg/L). A significant ($p < 0.01$) age-related effect on the whole blood Se concentration was observed. The highest percentage of individuals with adequate Se status and the lowest number of Se-depleted subjects were found among adults aged 21 to 40 years. In about 40 to 50% of adolescents and adults Se concentration was comprised within the suboptimal range of 60 to 80 µg Se/L.

Keywords: whole-blood selenium, Upper Silesia, adolescents, adults, the elderly

Introduction

Selenium, a nutritionally essential trace element, exerts its biological effect through over 35 selenoproteins identified in humans, some of which have important enzymatic functions [1-3]. These selenoproteins include several enzymes with antioxidant capacity such as glutathione peroxidase (GPx), phospholipid hydroperoxide glutathione peroxidase (PHGPx) and thioredoxin reductase (TR) [4]. Selenium deficiency is associated with a wide range of health outcomes such as an impairment of thyroid hormone metabolism, cancer, degenerative affections, cardiovascular diseases, viral infections, inflammatory conditions, immune function, chronic renal failure, and alcoholism [2, 3, 5-8]. However, whether optimal health depends upon adequate selenium status has yet to be determined.

Assessment of selenium status in humans is most frequently done by the determination of Se concentrations and/or activities of glutathione peroxidase in various biological materials such as whole blood, plasma, serum, erythrocytes, platelets, urine, toenails and hair [9,10]. The reference range for selenium in whole blood is comprised within the range of 80-250 µg/L [11, 12], while marginal and low marginal deficiency is found at concentrations less than 50 and 40 µg/L [11], respectively, whereas toxicity symptoms appear at levels higher than 500 µg/L [11, 13]. Whole blood and serum levels vary from one geographical area to another due to differences in daily intake of selenium, occupational exposure, alcohol intake, age, physical activity and general health status [14-19].

Plasma and serum concentrations are considered to a larger degree than erythrocytes, as being affected by recent changes in dietary intake of selenium. However, according to some authors [14] the relationship between selenium intake and body fluid concentrations seems to be

Corresponding author; e-mail: b.klapcinska@awf.katowice.pl

more linear for whole blood than for serum.

The available data on the Se status of the Polish population refers mostly to inhabitants of the northern and central part of the country [20-25], whereas only limited data exists concerning blood Se levels of a population living in a heavily industrialized southern part of the country [26-28]. Therefore, the present study was aimed at the evaluation of the age- and gender-dependent differences in the whole blood selenium concentration of a large cohort of apparently healthy residents of Upper Silesia.

Experimental Procedures

Study Population

Between 1999 and 2005, 1380 individuals (925 male and 455 female) aged 10 to 105yr at the time of examination were recruited for this study. The subjects were divided into six groups according to age: the youngest were adolescents aged 10 to 15 (group I), and the oldest were centenarians (group VI). Except for centenarians who were mostly (95 of 132 individuals) inhabitants of central Poland, the remaining subjects were all living in Upper Silesia. From the total number of subjects, only eight centenarians lived in nursing homes and none of them was on total parenteral nutrition. The number of subjects, subdivided according to gender and age, is listed in Table 1. The data on whole blood selenium

concentrations have been collected from our studies performed within the framework of several projects accomplished according to protocols approved by the Local Ethics Committee. The study was performed in compliance with the guidelines of the Helsinki Declaration as revised in 1996 regarding the use of human subjects.

Laboratory Procedures

Blood was collected by venipuncture in test tubes containing heparin as an anticoagulant. After thorough mixing the aliquots of 200 μ l were transferred into heparinized Eppendorf tubes and kept frozen at -20°C until analysis. Selenium determinations according to Danch and Drózdź (29) were performed on a Perkin-Elmer LS-30 spectrofluorometer. Whole blood samples (200 μ l) were transferred to special borosilicate glass test tubes (26 x 300 mm) filled with 4 ml portions of perchloric acid diluted with double distilled water (DDW) at the ratio of 7:3 and then digested at temperature $<210^{\circ}\text{C}$ in a heating block (Meditherm TH-3) placed under a fume hood until the mineralizates were colourless or slightly yellow. Subsequently, a 0.5 ml-portion of DDW was added to each tube and heating was continued until white fumes were driven off. Then, the tubes were removed from the heating block, 1 ml-portion of 1.0 M hydrochloric acid was added and the tubes were placed into a boiling water

Table 1. Whole blood selenium (Se, $\mu\text{g/L}$) in different population subgroups (M-male, F-female)

Group	Age (years)	Gender	N	Whole blood Se, $\mu\text{g/L}$	SD	Minimum	Maximum
I	10-15	M	119	59.8*	15.4	26.6	91.9
		F	45	58.0	12.4	30.7	84.8
II	16-20	M	96	60.9*	14.7	30.9	93.1
		F	31	55.0	12.8	34.0	89.4
III	21-40	M	606	69.2	16.8	23.3	165.6
		F	131	63.8	16.7	26.4	111.6
IV	41-69	M	69	65.5	17.3	29.0	107.4
		F	102	67.1	18.1	27.1	151.7
V	70-99	M	16	51.9*	7.6	33.4	62.1
		F	33	55.4	15.7	27.1	94.2
VI	≥ 100	M	19	51.2*	19.5	28.2	91.9
		F	113	44.9 [#]	19.0	13.5	116.6
I-VI		M	925	66.2	17.0	23.3	165.6
I-VI		F	455	58.1 ^{&}	18.9	13.5	151.7
I-VI		M/F	1380	63.5	18.1	13.5	165.6

Note: *-significantly different ($p < 0.05$) from the respective values in male subjects aged 21-40 yr (group III); [#]-significantly ($p < 0.01$) different from the respective values in female subjects aged 41-69 yr (group IV); [&]-significantly ($p < 0.01$) different from male subjects

bath for 1 hr in order to allow conversion of any Se(VI) to Se(IV). After cooling to room temperature, 2.5 ml of EDTA (9 g/L) plus hydroxylamine (15 g/L) reagent and 2 drops of bromocresol red indicator were added to each test tube. The pH was then adjusted to 1.5-2.0 by titrating with ammonia solution (1:1) to pale rose colour. The following steps, i.e. incubation with freshly prepared 2,3-diaminonaphthalene (4 g/L in 0.1 M HCl) at 40°C for 30 min. and extraction of the selenol formed with 5 ml cyclohexane were performed in the dark. Finally, the fluorescence of the extracts was read at 365 nm (excitation) and 520 nm (emission). At each series of determinations selenium methionine (SIGMA) was used as an external standard. The same procedure was applied with blanks (200 µl DDW) and standard selenium methionine solutions containing 10 to 150 µg Se/L to obtain the calibration curve. The blanks and standards were included in each set of selenium determinations. The limit of detection was stated at 0.45 ng Se in the sample, the intra-assay and inter-assay coefficients of variation (CV) for whole blood were, respectively, 4.8% (n=50) and 4.9% (n=15).

Statistics

The results are expressed as means \pm SD. The check for normal distribution was performed with the Kolmogorov-Smirnov test. Group differences were identified with one- or two-way ANOVA after checking for homogeneity of variances with the Levene test, followed by the Tukey comparisons where appropriate. Independent variables included gender and age. All statistical analyses were performed using STATISTICA 5.0 (StatSoft, Inc. 1995) software. The level of significance was set at $p < 0.05$.

Results

Concentration of selenium in whole blood was found to be distributed normally with an average value of 63.5 ± 18.1 µg Se/L (min. 13.5 and max. 165.6 µg/L). Mean Se concentration in male subjects (66.2 ± 17.1 µg/L) taken as a whole was significantly ($p < 0.01$) higher as compared to that recorded in females (58.1 ± 18.9 µg/L). A significant ($p < 0.01$) age-related effect on the whole blood Se concentration was observed. Selenium concentrations in whole blood for different age groups, subdivided according to gender, are presented in Table 1. The highest Se levels were recorded in men aged 20 to 40 (group III) and in women aged 41-60 (group IV). A percent distribution of subjects (both male and female) with low marginal (<40 µg/L) or marginal (40-60 µg/L) Se deficiency, suboptimal (60-80 µg/L) and adequate (>80 µg/L) selenium status in separate age groups is presented in Fig.1. Additionally, the frequency distribution of whole blood Se is presented separately for the whole group of male (Fig.2) or female (Fig.3) subjects.

Discussion

Our results have evidenced inadequate Se status in a vast majority of the locally living population sample. Only in about 12% of male and in 10% female subjects, taken as a whole, the blood concentration of Se exceeded the lowest limit of the adequacy range (80 µg/L) [11,12], whereas in almost 40% of males and 30% of females Se content in blood was comprised within the suboptimal range (60-80 µg/L), while in the remainder it was even lower. The data obtained in the present study are very close to those reported by other authors in populations living in Lower Silesia [26], a central part of the country

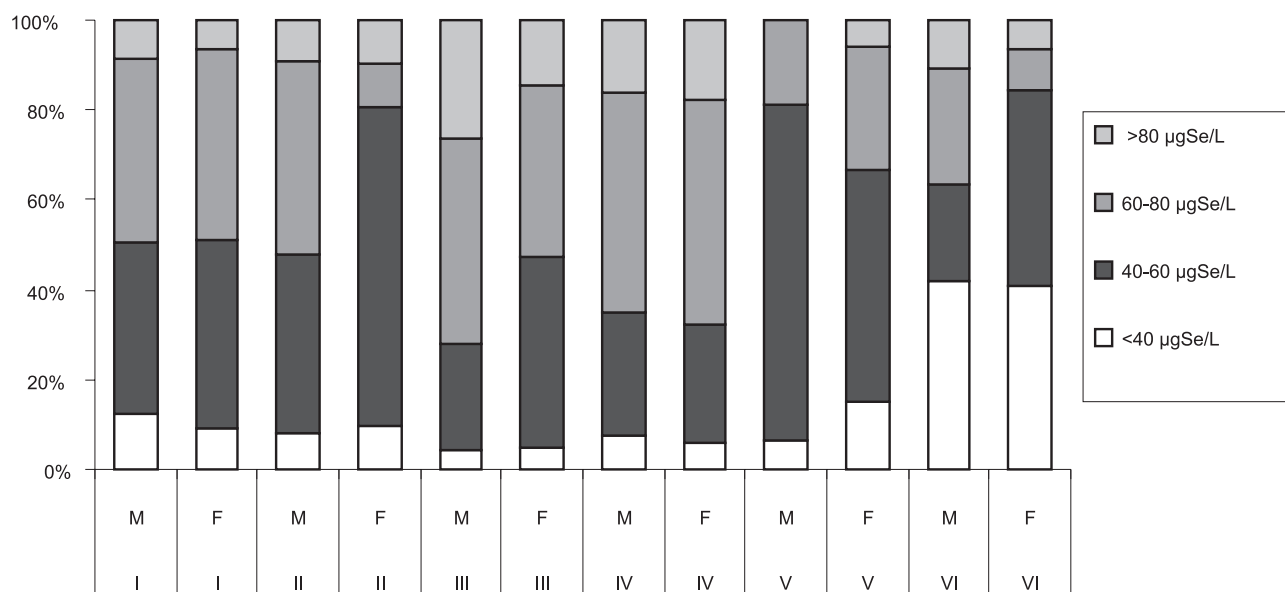


Fig. 1. Percent distribution of subjects, both male (M) and female (F) from groups I – VI, with low marginal (<40 µg/L) or marginal (40-60 µg/L) Se deficiency and suboptimal (60-80 µg/L) or the adequate Se concentration (>80 µg/L).

[20-24] and central Pomerania [25]. Similar observations have been made in most European inhabitants, although significant differences have occurred between different countries [30]. Interestingly, suboptimal blood Se levels were also reported in healthy adults in Slovakia [31] and the Czech Republic [32], which are the closest neighbouring countries.

Whole blood Se levels reported in our study appeared to be age-dependent ($p < 0.01$), and a gradual decline with age was observed in the elderly and the oldest individuals, with the highest percentage of Se-depleted ($< 40.0 \mu\text{g Se/L}$) subjects found among centenarians, both men and women. In this respect, our data are in agreement with those reported by other authors. A marginal Se deficiency ($< 69 \mu\text{g/L}$) was revealed in an aged Finnish population by Kivela et al. [33], in healthy elderly (aged 65 yr and over) British by Bates et al. [34], as well as by Savarino [35] in healthy Northern Italian nonagenarians and centenarians. A contrary trend, i.e. an increase with age observed among our adolescent subjects (groups I and II), supports the observations of Bates et al. [36] collected during a national-scale survey among British young people aged 4 to 18. Literature data on selenium status in infants and children [37] indicates that the median serum Se concentrations steadily decrease from birth to a minimum concentration at the age of 4 months. Blood selenium concentrations in children over 1 year of age increases progressively until maturity [13], and differences observed in different countries seem to re-

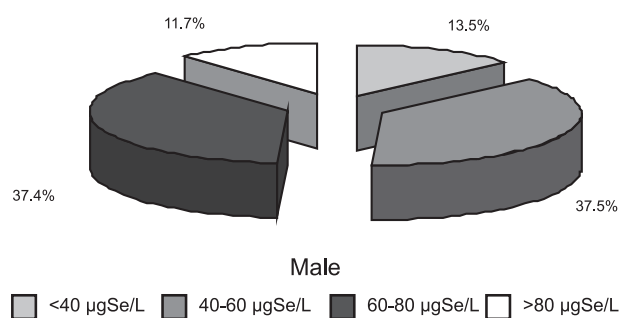


Fig. 2. Frequency distribution of whole-blood Se concentration in male subjects.

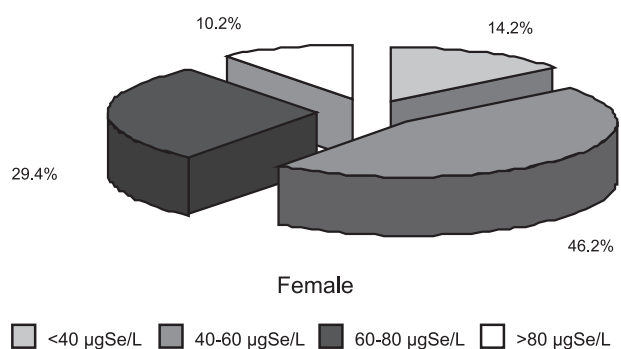


Fig. 3. Frequency distribution of whole-blood Se concentration in female subjects.

flect regional variations in Se availability. Whole blood, plasma or serum Se levels are usually higher in healthy adults [19] than in adolescents and the elderly. The most plausible cause of the age-related differences in selenium concentrations may be attributable to the changes in composition of a diet eaten during various periods of the whole life-span, which is also dependent on some other factors such as socio-economic status, food habits, lifestyle or dwelling place [18, 19, 34, 36, 38].

The major determinant of Se status in humans is the level of dietary intake, which mostly depends on the amount of selenium bound in feeds, edible plants and human foods that vary according to the concentration and biological availability of Se in the soil. Dietary Se intakes in Poland have fallen over the last 20 years [22]. It is noteworthy that concentrations of Se, as determined in the late 1980s, e.g. in bakery products ($76 \mu\text{g/kg}$) [39] have, in 2002, decreased to significantly lower levels (22.9 to $53.0 \mu\text{g/kg}$) [40]. The main causes of these changes are low Se content in soil and in domestic agricultural products and a low ratio of the imported high-Se wheat and corn from North America to the low-Se domestic grain [41]. It has been estimated that the mean Se intake of the Polish population has changed from $110 \mu\text{g}$ daily with homemade diets and $145 \mu\text{g/d}$ with canteen-made rations in 1989-90 [42] to 30 - $40 \mu\text{g Se/d}$ in 2003 [22]. Functional consequences of this decline have not been recognized as subjects examined within the frame of our study were apparently healthy with no overt symptoms of Se deficiency, which may imply that a suboptimal level within the range of 60 to $80 \mu\text{g Se/L}$ may be sufficient for maintaining selenium homeostasis. Therefore, it seems possible, as suggested by Daniels [43], "to advance a range of theoretical arguments that suboptimal Se status may have an effect on health, but evidence of a direct relationship to health outcomes is very limited." A similar suggestion has been put forward previously by Alftan and Nève [44]. It should be stressed, however, that it is difficult to correlate biochemical deficiency with clinical symptoms because Se has many roles in metabolism and the existence of a synergism between different micronutrients (such as vitamin E and Se) has also to be taken into account [45]. Therefore, a final settlement over the acceptable level of whole blood selenium would be made only if the Se status within the suboptimal range of 60 to 80 ng/ml can actually be excluded from other disease-predisposing etiological factors.

In conclusion, a sub-optimal selenium status has been identified in a large cohort of apparently healthy residents of the Upper Silesia region. The results do suggest that the sample may not be meeting physiological requirements for selenium ($55 \mu\text{g/d}$) as recommended by the most recent US RDA [46]. A significant ($p < 0.01$) age-related effect on the whole blood Se concentration has been observed. As compared to adult subjects aged 21 to 40 who presented a relatively high blood Se level, the highest number of Se-depleted individuals was found among centenarians and the elderly.

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