EFFECTS OF AGE, BMI, SMOKING AND CONTRACEPTION ON LEVELS OF Cu, Se AND Zn IN THE BLOOD OF THE POPULATION IN THE CZECH REPUBLIC

Beneš B., Spěváčková V., Šmíd J., Batáriová A., Čejchanová M., Zítková L.
National Institute of Public Health, Prague, Czech Republic

SUMMARY

Variations in the levels of Cu, Se and Zn in blood were analyzed in relation to gender, age, BMI, smoking and hormonal contraception. The blood samples were collected from 3,207 blood donors (2,362 men, 845 women) during years 1996–2003. Blood concentrations of these elements were in the same range as those found for populations of other European countries. Significant differences exist between men and women, in blood concentration, for Cu (840 μg $Cu.l^{-1}$ vs. 970 μg $Cu.l^{-1}$, $p<0.01$) and Zn (6,780 μg $Zn.l^{-1}$ vs. 6,235 μg $Zn.l^{-1}$, $p<0.01$) only. The level of Cu in relation to age is increasing in men, but decreasing in women. The level of Se in relation to age increases regardless of sex. Concentrations of Zn rise with age in women group, only. Positive correlation was found between BMI and level of Cu only for group of men. For Se levels and BMI differences exist only in men namely between groups <20 and >35 (82 μg $Se.l^{-1}$ vs. 92 μg $Se.l^{-1}$). Slight positive correlations exist between Zn concentration and BMI in women only. The significant differences were found between smokers and non-smokers for Cu in men (850 μg $Cu.l^{-1}$ vs. 830 μg $Cu.l^{-1}$) and Se (81 μg $Se.l^{-1}$ vs. 84 μg $Se.l^{-1}$). The smoking has not significant influence on concentration of Zn in the blood. The hormonal contraception significantly increases the concentration of Cu in blood (920 μg $Cu.l^{-1}$ vs. 1,270 μg $Cu.l^{-1}$, $p< 0.01$). The levels of Se and Zn in blood are not influenced using hormonal contraception.

Key words: trace elements, blood, age, BMI, smoking, contraception

Address for correspondence: B. Beneš, National Institute of Public Health, Šrobárova 48, 100 42 Prague 10, Czech Republic. E-mail: bbenes@szu.cz

INTRODUCTION

The importance of trace elements for human organism is indisputable (1, 2). Trace elements such as copper (Cu), selenium (Se) and zinc (Zn) are accepted as essential for optimal human health because of their diverse well-documented metabolic function. They have catalytic, structural and regulatory roles and interact with different macromolecules such as enzymes, hormones, proteins and other biological molecules (3, 4). The knowledge of normal concentrations of these elements in healthy occupationally unexposed population is of relevance not only to the public health regulatory authorities in determination, for example, exposure limits, but also for the prevention and treatment of some disorders caused by deficiency of essential elements or toxicity of others (5–8). The normal values are crucial but may be also influenced by various factors. It is particularly true for elements, which are not homeostatic controlled (9, 10). Among the interfering factors are gender, age, smoking status, alcohol consumption, ethnic origin, weight, medication, climate condition and last but not least life style, eating habits, residential area and quality of the environment (11, 12). The overall effect of all these factors on the normal concentrations of trace elements in the population is very difficult or almost impossible to determine. Therefore we attempted to determine the roles of some basic factors as gender, age, weight, smoking and use of female oral contraception on levels of Cu, Se and Zn in whole blood (13, 14). The selected elements are considered to have particular relevance to human health, environment and therapeutic purposes, and reliable data are therefore urgently in need. Previous epidemiological studies of the trace elements status have been carried out in different European countries, mainly in west and northern ones (15). However in the Central Europe and particularly in the Czech Republic are the studies dealing with trace elements and factors of their prevalence and effect very scarce. The present study is an integral part of the ongoing project " System of Monitoring the Environmental Impact on the Population Health" in the Czech Republic.

MATERIAL AND METHODS

Collection of Samples

Concentrations of trace elements (Cu, Se and Zn) were determined in samples of whole blood collected from 3,207 blood donors (2,362 men and 845 women) of average age 33 years (men 33.6 and women 32.4). Blood samples were collected consecutively from 1996 to 2003 year in the transfusion centers of the districts Benešov, Plzeň, Ústí nad Labem and Žďár nad Sázavou. The venipuncture of the individuals was carried out in the

Table 1. *Blood Cu, Se and Zn levels (μg . l^{-1}) from the Czech population, according to gender*

	Sex	N	Avg	SD	GM	Me	$Kv_{0.25}$	$Kv_{0.75}$	$Kv_{0.95}$	p
Cu	M	2,362	845	120	836	840	770	920	1,037	< 0.01
	F	845	1,029	256	999	970	840	1,180	1,510	
Se	**M**	2,146	84	20	81	84	72	96	117	none
	F	764	84	18	81	84	73	95	113	
Zn	**M**	1,778	6,945	1,244	6,842	6,780	6,163	7,498	9,102	< 0.01
	F	606	6,413	1,192	6,315	6,235	5,640	6,960	8,443	

M = male, F = female, N = number of subjects, Me = median, p = statistical significance, Avg = average

Table 2. *Blood Cu, Se and Zn levels (μg . l^{-1}) from the Czech population, according to age*

Age		< 20		21–30		31–40		41–50		>50	
	sex	N	Me	N	Me	N	Me	N	Me	N	Me
Cu	M	46	790	810	830	770	840	429	860	14	870
	F	48	1,040	259	980	259	950	161	950	12	990
Se	M	46	79	810	83	770	82	429	84	14	93
	F	48	73	259	85	259	83	161	85	12	87
Zn	M	46	6,065	809	6,470	770	6,500	428	6,585	14	6,685
	F	48	5,515	259	5,850	259	6,010	161	6,160	12	6,170

M = male, F = female, N = number of subjects, Me = median

morning after 12-h fasting into collection vessels (S-Monovette, Sarsted 02.1065.400) using silicon-coated needles. Blood samples were stored at –20 ^{0}C. Of 845 women investigated 209 ones were on hormonal contraceptives for more than one year. From the total count of subjects 1,144 were smokers (870 men, 274 women).

Analytical methods

Mineralization. Blood samples (2 ml) were mineralized in microwave system (Milestone 1200 Mega, U.S.A.) by means of the mixture 10 ml concentrated HNO_3 + 1 ml 30% H_2O_2 (Suprapur Merck, Germany). After mineralization the acidic mixture was evaporated to the volume of about 0.2 ml and the volume of the solution was made up to 10 ml with demineralized water.

The analyses. Individual elements were determined in the whole blood by means of atomic absorption spectrometry-AAS (16, 17). Cu and Zn were determined by flame AAS (flame air-acetylene), instrument Perkin Elmer 3300 (U.S.A.). Total Se concentration was determined by the technique of hydride generation with HCl and $NaBH_4$ as reduction agents, using instrumentation Perkin Elmer 3300 + FIAS 400 (U.S.A.). For all elements the standards were prepared from standard solution AAS (Merck, Germany). The accuracy was checked with standard reference materials (Control Material Seronorm Whole Blood 40407 and Serum 704121, Nycomed, Norway). Check and blank samples were prepared and measured simultaneously with every series of analyzed samples. Statistical evaluation was made with χ^2, Student's t-test and Kruskal-Wallis test and by means of programs STATISTICA 7 CZ (Statsoft Inc. U.S.A.) and ANOVA. Statistical significance was set at $p < 0.05$ level.

RESULTS

The results of determinations of Cu, Se and Zn in the whole blood are presented in Tables and Figures as medians (Me), μg.l^{-1}. To analyze the effect of age, the study subjects were divided into five groups i.e < 20 years, 21–30 years, 31–40 years, 41–50 years, > 50 years. To study the effect of BMI (body mass index) five categories as follows were compared: i.e. < 20, 20–25, 25–30, 30–35 and >35.

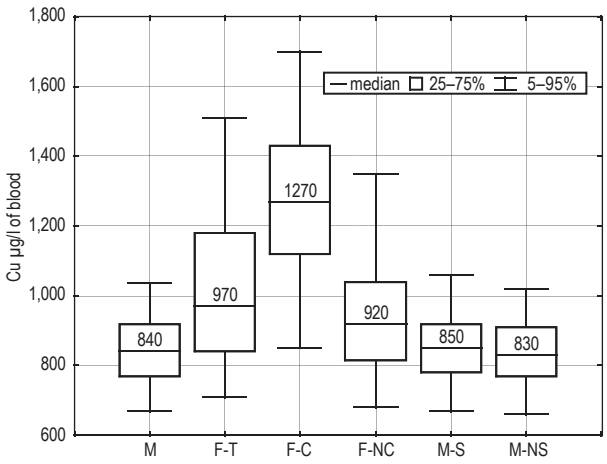

Fig. 1. *Blood Cu levels according to gender, smoking status and hormonal contraception.*

M = male, F-T = female total, F-C = female with contraception, F-NC = female without contraception, M-S = male smokers, M-NS = male non-smokers)

Table 3. *Blood Cu, Se and Zn levels (μg . l^{-1}) from the Czech population, according to BMI*

BMI		< 20		20–25		25–30		30–35		>35	
	sex	N	Me	N	Me	N	Me	N	Me	N	Me
Cu	M	31	780	802	820	1,000	840	217	870	21	940
	F	71	940	401	960	174	950	61	980	26	1,010
	T	102	870	1,203	850	1,174	850	278	880	47	980
Se	M	29	82	803	83	999	83	218	85	21	92
	F	71	79	405	84	175	84	60	81	26	82
	T	100	80	1,208	83	1,174	83	278	84	47	84
Zn	M	31	6,480	803	6,470	1,000	6,510	217	6,675	21	6,950
	F	71	5,950	405	5,810	173	6,145	60	5,820	26	6,325
	T	102	6,165	1,208	6,230	1,173	6,440	277	6,510	47	6,480

BMI = body mass index, M = male, F = female, T = total, N = number of subjects, Me = median

Copper

The values of the blood Cu concentrations in relation to gender and age are summarized in Table 1–2 and in Fig.1–2, resp. Statistically significant differences were observed between men and women (840 μg Cu.l^{-1} vs. 970 μg Cu.l^{-1}, $p < 0.01$). In relation to age Cu levels have significantly increasing tendency in men ($p < 0.01$) (Fig. 2). On the contrary, Cu in women has decreasing tendency, surprisingly, on limit of significance (Fig 3). Positive correlation was found between BMI and concentration of Cu for group of men ($p < 0.01$) (Fig. 4, Table 3). The differences in the blood Cu, between smokers and non-smokers are statistically significant in men ($p < 0.01$, 850 μg Cu.l^{-1} vs. 830 μg Cu.l^{-1}) (Fig. 1). It is of interest, that in the group of women these differences are not significant. Women on hormonal contraception have considerably

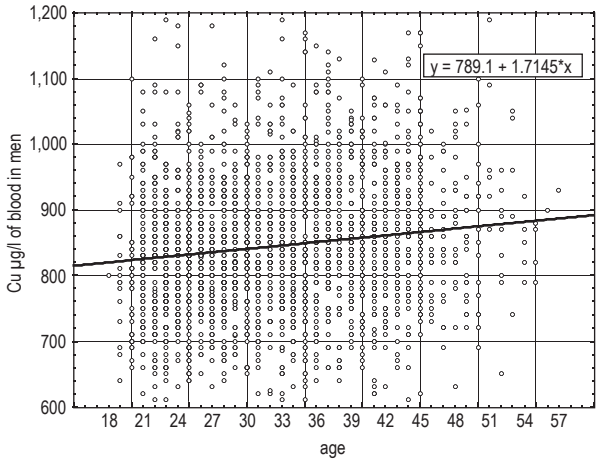

Fig. 2. *Relationship between age and level of Cu in blood of men.*

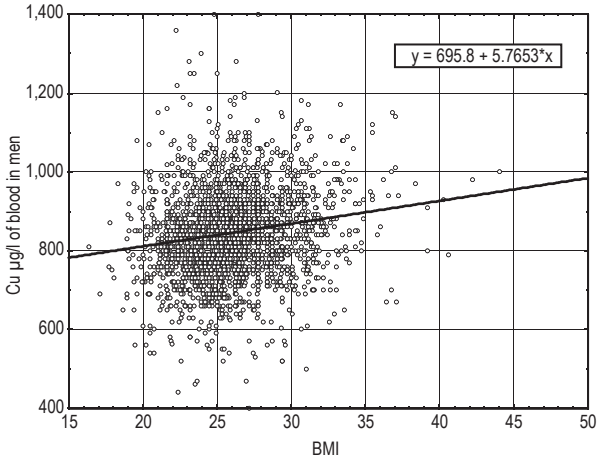

Fig. 4. *Relationship between BMI and level of Cu in blood of men.*
BMI = body mass index

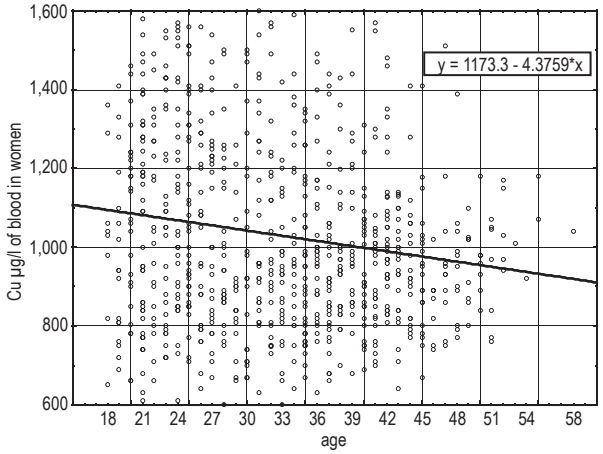

Fig. 3. *Relationship between age and level of Cu in blood of women.*

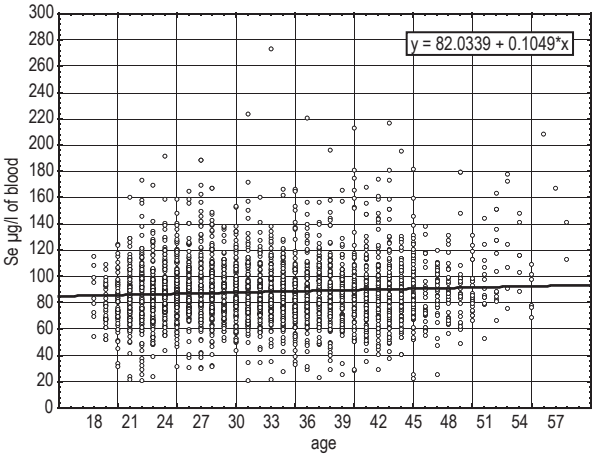

Fig. 5. *Relationship between age and level of Se in blood of Czech population.*

higher concentration of Cu in blood (1,270 μg $Cu.l^{-1}$) compared to women without it (920 μg $Cu.l^{-1}$, $p < 0.001$) (Fig. 1).

Selenium

No significant differences were observed in the blood Se concentration between men and women (84 μg $Se.l^{-1}$) (Table 1). Statistically significant differences were detected only between age group < 20 and group > 50 ($p < 0.01$); no influence of gender was observed (Table 2). As far as Se concentrations in blood is concerned slight positive correlations, on the level of significance (< 0.05) were found with regards to age (Fig. 5). In case of BMI a significant difference ($p < 0.05$) in Se levels in men between groups < 20 (82 μg $Se.l^{-1}$) and >35 (92 μg $Se.l^{-1}$) was ascertained (Table 3). Smoking status influenced concentration of Se in blood. In general the smokers have significantly ($p < 0.01$) lower Se concentration in blood than non-smokers (81 μg $Se.l^{-1}$ vs. 84 μg $Se.l^{-1}$). The hormonal contraception did not influence concentrations of Se in the blood.

Zinc

The concentration of Zn in the blood (Table 1) is significantly higher ($p < 0.01$) in men than in women (6,780 μg $Zn.l^{-1}$ vs. 6,235 μg $Zn.l^{-1}$) (Table 1). Slight positive correlation between Zn blood concentration and age ($p < 0.05$) and BMI, respectively was found only in women. (Table 2, Fig. 6. Table 3, Fig. 7).The analogous tendency was also found in men, but it was surprisingly insignificant. No correlation between Zn blood level, hormonal contraception and smoking, respectively, was detected. Analysis has shown no significant correlation between blood concentration of Cu and Zn too.

DISCUSSION

Concentrations of Cu, Se and Zn according to Gender, Age and Hormonal Contraception

Blood Cu concentrations of similar values were generally found in other European countries as well (18, 19). The levels of Cu were influenced by gender significantly. As it has been already shown in some previous studies women have markedly higher Cu values than men (11, 16, 20). These differences are evidently due to hormonal and estrogene status, respectively. This fact is supported by evidence that levels of Cu in women using hormonal contraception (with estrogene component) are among the highest. It is possible to suppose that estrogens induce ceruloplasmin synthesis in the liver, which leads to an increase of blood Cu concentration (21). This presumption is also supported by the observation, that concentration of Cu blood level in young girls (age 10 years, without hormonal activity), when compared with levels in boys of the same age (16, 18) do not differ. The relationship between age and Cu blood level has an increasing tendency in men and decreasing in women. It is possible to explain it by decreasing levels of sex hormones and their declining activity with increasing age. So, for example in elder men, the relatively lower concentration of Cu in blood could be related to decrease of the testosterone level with age.

Concentrations of Se presented in this study do not differ from data published in other European regions, analogous to the Czech Republic, in respect of their geological, geographical and demographic circumstances (22). No significant differences were observed in the blood Se concentrations between men and women. It is in contradiction with some previous reports, which reported significantly higher values in men than in women (23, 24). Our results are similar to already published data (25, 26). Similar inconsistency was found in relationship between Se blood levels and age. In our case, identically with data already published (24, 3, 13) levels of Se have significantly increasing tendency with age. On the contrary Wasowicz (27) found that serum Se concentration remained constant in the range 16–60 years. Lloyd (28) even reports, that concentration of Se is decreasing. Previous investigation on age related differences in blood Se could be in dispute for the age ranges used in those studies were either too small or too large (13). Similarly the dimension of the single groups has considerable influence, also. Hormonal contraception has not any influence on concentration of Se in blood.

The values of Zn in blood of the Czech population are slightly higher in men than in women. The range values for both sexes were similar to those found in other European population groups (11, 29). This is given apparently by homeostatic character of Zn, which in our study rises significantly with age only in women. In men differences are insignificant. Comparison with other reports is very difficult. Grandjean (22) found that serum Zn decreases

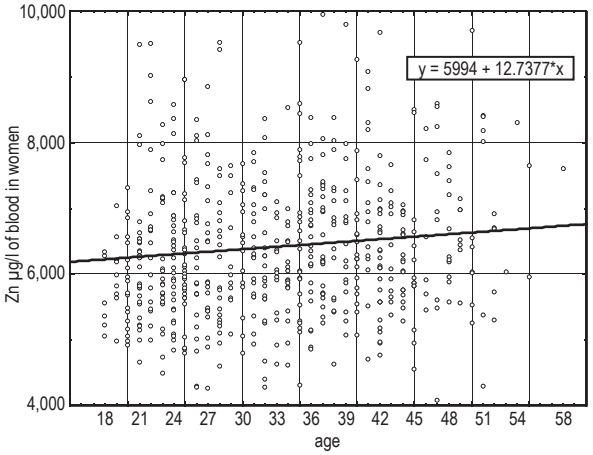

Fig. 6. *Relationship between age and level of Zn in blood of women.*

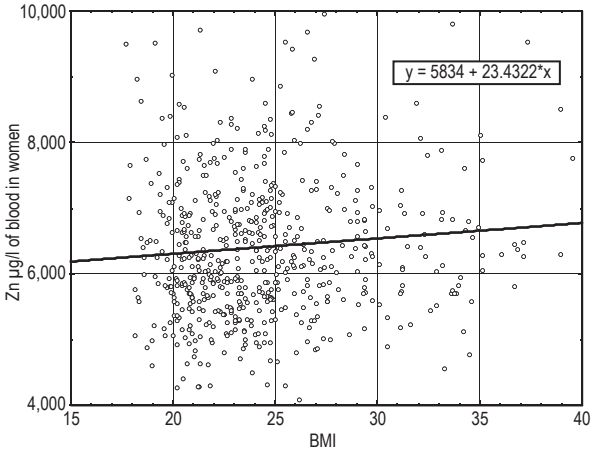

Fig. 7. *Relationship between BMI and level of Zn in blood of women.*

with age in men, while others observed no significant differences (30). The rise of concentrations of some trace elements in blood with age is hypothetically explained by the free radical theory of aging (29, 31). Unfortunately, this theory cannot explain many discrepancies in metabolism of trace elements. For example, Cu in men increases with age, but in women it decreases, and the same effect is observed in Zn. It may be presumed, that in case of Cu its levels are again influenced hormonally. Contraception has not any influence on concentration of Zn in blood of the Czech population.

Levels of Cu, Se and Zn in Relation to Smoking Status and BMI

Concentration of Cu in blood of men is increased slightly with smoking, but in women there was no significant difference between smokers and non-smokers. The reason for the latter could be a low number of smoked out cigarettes in women. Similar results were reported by Dubick (32) and other authors (33) did not confirm the influence of smoking on Cu blood levels too.

Levels of Se in blood were influenced by tobacco smoking significantly. The smokers had lower levels of Se than non-smokers. These findings are in agreement with previous reports (34, 35). It has been reported that smokers, generally, consume less dietary selenium compared to non-smokers and also selected diet is low in nutrient density (36). So the decreased selenium content in blood of smokers was probably due to low dietary selenium intake by smokers rather than by smoking itself (25).

In our study the concentration of Zn in the blood is not influenced by tobacco smoking significantly. These results differ from findings of Lopez (3) who found higher Zn content in men smokers.

Blood Cu concentrations in men increased proportionally with BMI. In the group of women no significant influence of BMI on the level of Cu was observed.

There was also no association between BMI and levels of Se in blood of women. The differences exist only in men whose BMI ranges between < 20 and > 35 probably owing to higher food intake and consequently higher intake of Se.

The concentrations of Zn increase slightly in relation with BMI in women while in men the noted increase was insignificant.

The present study provided hitherto missing items for evaluation of the trace element status in Czech population, concerning to some pathological conditions as are e.g. cardiovascular or oncogenous diseases.

Acknowledgements

The authors thank the field workers and technicians from the District Public Health Institutes of Benešov, Plzeň, Ústí nad Labem and Žďár nad Sázavou for sample collection.

REFERENCES

1. Bogden JD, Kleevy LM. Clinical nutrition of the essential trace elements and minerals. New York: Humana Press; 2000.
2. Neve J, Chapuis P, Lamand M, editors. Therapeutic uses of trace elements. New York: Plenum Press; 1996.
3. Lopez PA, Santos MC, Vicente L, Rodrigues MO, Pavao ML, Neve J, Viegas-Crespo AM. Trace Elements Status (Se, Cu, Zn) in healthy Portuguese subjects of Lisboa population: a reference study. Biol Trace Elem Res. 2004;101(1):1–17.
4. First symphosium on human health related aspects of selenium research in Europe, November 27, 1999, Brussels, Belgium [abstracts]. J Trace Elem Med Biol. 2000;14:116–21.
5. Buchancová J, Knižková M, Hýllová D, Vrlík M, Meško D, Klimentová G, Gáliková E. Content of the selected trace elements (Al, As, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Zn) in blood, urine and hair of blood donors without occupational exposure to these metals. Cent Eur J Public Health. 1994;2(2):82–7.
6. Puklová V, Batariová A, Černá M, Kotlík B, Kratzer K, Melicherčík J, Ruprich J, Řehůřková I, Spěváčková V. Cadmium exposure pathways in the Czech urban population. Cent Eur J Public Health. 2005;13(1): 11–9.
7. Bencko V, Cikrt M, Lener J. Toxic metals in the living and working environment. Prague: Grada Publishing; 1995. (In Czech)
8. Taylor A. Detection and monitoring of disorders of essential trace elements. Ann Clin Biochem. 1996 Nov;33 (Pt 6):486–510.
9. Milne DB. Copper intake and assessment of copper status. Am J Clin Nutr. 1998 May; 67(5 Suppl):1041S–5S.
10. Hambidge M. Human zinc deficiency. J Nutr. 2000 May;130(5 Suppl):1344S–9S.
11. Versieck J, Cornelis R. Trace elements in human plasma or serum. Boca Raton (FL): CRC Press; 1989.
12. Lachili B, Faure H, Arnaud J, Richard MJ, Benlatreche C, Favier A, Roussel AM. Blood micronutrients in Algeria, relationships with sex and age. Int J Vitam Nutr Res. 2001 Mar;71(2):111–6.
13. Robberecht H, Deelstra H. Factors influencing blood selenium concentration values: a literature review. J Trace Elem Electrolytes Health Dis. 1994 Dec;8(3–4):129–43.
14. Bureau I, Anderson RA, Arnaud J, Raysiguier Y, Favier AE, Roussel AM. Trace mineral status in post menopausal women: impact of hormonal replacement therapy. J Trace Elem Med Biol. 2002;16(1):9–13.
15. Mussalo-Rauhamaa H, Kantola M, Seppanen K, Soininen L, Koivusalo M. Trends in the concentrations of mercury, copper, zinc and selenium in inhabitants of north-eastern Finish Lapland in 1982–1991. A pilot study. Arctic Med Res. 1996;55(2):83–91.
16. Beneš B, Spěváčková V, Šmíd J, Čejchanová M, Černá M, Šubrt P, Mareček J. The concentration levels of Cd, Pb, Hg, Cu, Zn and Se in blood of the population in the Czech Republic. Cent Eur J Public Health. 2000 May;8(2):117–9.
17. White MA, Sabbioni E. Trace element reference values in tissues from inhabitants of the European Union. X. A study of 13 elements in blood and urine of a United Kingdom population. Sci Total Environ. 1998 May 27;216(3):253–70.
18. Krause C, Bobisch W, Becker K, Bernigau W, Hoffmann K, Nöllke P, Schulz C, Schwabe R, Seiwert M, Thefeld W. Umwelt-Survey 1990/92, Band Ia: Studienbeschreibung und Human-Biomonitoring: Deskription der Spurenelementgehalte in Blut und Urin der Bevölkerung der Bundesrepublik Deutschland. WaBoLu-Hefte 1/1996. Berlin: Institut für Wasser-, Boden- und Lufthygiene des Umweltbundesamtes; 1996.
19. Poulsen OM, Christensen JM, Sabbioni E, Van der Venne MT. Trace element reference values in tissues from inhabitants of the European Community. V. Review of trace elements in blood, serum and urine and critical evaluation of reference values for the Danish population. Sci Total Environ. 1994 Jan 25;141(1–3):197–215.
20. Wang WC, Heinonen O, Makela AL, Makela P, Nanto V, Branth S. Serum selenium, zinc and copper in Swedish and Finnish orienteers. A comparative study. Analyst. 1995 Mar;120(3):837–40.
21. Martin-Lagos F, Navarro-Alarcon M, Terres-Martos C, Lopez-Garcia de la Serrana H, Perez-Valero V, Lopez-Martinez MC. Zinc and copper concentrations in serum from Spanish women during pregnancy. Biol Trace Elem Res. 1998 Jan;61(1):61–70.
22. Grandjean P, Nielsen GD, Jorgensen PJ, Horder M. Reference intervals for trace elements in blood: significance of the risk factors. Scand J Clin Lab Invest. 1992 Jun;52(4):321–37.
23. McMaster D, Bell N, Anderson P, Love AH. Automated measurement of two indicators of human selenium status, and applicability to population studies. Clin Chem. 1990 Feb;36(2):211–6.
24. Kafai MR, Ganji V. Sex, age, geographical location, smoking, and alcohol consumption influence serum selenium concentrations in the USA: third national health and nutrition examination survey, 1988–1994. J Trace Elem Med Biol. 2003;17(1):13–8.
25. Swanson CA, Longnecker MP, Veillon C, Howe M, Levander OA, Taylor PR, McAdam PA, Brown CC, Stampfer MJ, Willett WC. Selenium intake, age, gender, and smoking in relation to indices of selenium

status of adults residing in a seleniferous area. Am J Clin Nutr. 1990 Nov;52(5):858–62.
26. Brtková A, Magálová T, Babinská K, Bederová A. Serum selenium levels in Slovak population. Biol Trace Elem Res. 1994 Oct–Nov;46(1–2):163–71.
27. Wasowicz W, Zachara BA. Selenium concentrations in the blood and urine of a healthy Polish sub-population. J Clin Chem Clin Biochem. 1987 Jul;25(7):409–12.
28. Lloyd B, Lloyd RS, Clayton BE. Effect of smoking, alcohol, and other factors on the selenium status of a healthy population. J Epidemiol Community Health. 1983 Sep;37(3):213–7.
29. Madaric A, Ginter E, Kadrabova J. Serum copper, zinc and copper/zinc ratio in males: influence of aging. Physiol Res. 1994;43(2):l07–11.
30. Takikawa S at al. Changes in serum Zn, Cu, Se, and Mn levels in patiens with chronic liver diseases and hepatocellular carcinoma. J Clin Biochem Nutr. 1990;8:153–64.
31. Harman D. Free radicals in aging. Mol Cell Biochem. 1988 Dec;84(2):155–61.
32. Dubick MA, Keen CL. Influence of nicotine on tissue trace element concentrations and tissue antioxidant defense. Biol Trace Elem Res. 1991 Nov;31(2):97–109.
33. Kocyigit A, Erel O, Gur S. Effects of tobacco smoking on plasma selenium, zinc, copper and iron concentrations and related antioxidative enzyme activities. Clin Biochem. 2001 Nov;34(8):629–33.
34. Ellis NI, Lloyd B, Lloyd RS, Clayton BE. Selenium and vitamin E in relation to risk factors for coronary heart disease. J Clin Pathol. 1984 Feb;37(2):200–6.
35. Ellingsen D, Thomassen Y, Efskind J, Aaseth J. Blood selenium in smokers and non-smokers in Norway. J Trace Elem Exp Med. 2004;17(4):183–87.
36. Fehily AM, Phillips KM, Yarnell JW. Diet, smoking, social class, and body mass index in the Caerphilly Heart Disease Study. Am J Clin Nutr. 1984 Oct;40(4):827–33.

Received October 15, 2005
Received in revised form and accepted August 22, 2005