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

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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 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

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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
morning after 12-h fasting into collection vessels (S-Monovette, Sarsted 02.1065.400) using silicon-coated needles. Blood samples were stored at –20°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 HNO₃ + 1 ml 30% H₂O₂ (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 NaBH₄ 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 χ², 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.](image)

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

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

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Avg</th>
<th>SD</th>
<th>GM</th>
<th>Me</th>
<th>Kᵥ₂₅⁺</th>
<th>Kᵥ₇₅⁺</th>
<th>Kᵥ₉₅⁺</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>M</td>
<td>2,362</td>
<td>845</td>
<td>120</td>
<td>836</td>
<td>840</td>
<td>770</td>
<td>920</td>
<td>1,037</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>845</td>
<td>1,029</td>
<td>256</td>
<td>999</td>
<td>970</td>
<td>840</td>
<td>1,180</td>
<td>1,510</td>
</tr>
<tr>
<td>Se</td>
<td>M</td>
<td>2,146</td>
<td>84</td>
<td>20</td>
<td>81</td>
<td>84</td>
<td>72</td>
<td>96</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>764</td>
<td>84</td>
<td>18</td>
<td>81</td>
<td>84</td>
<td>73</td>
<td>95</td>
<td>113</td>
</tr>
<tr>
<td>Zn</td>
<td>M</td>
<td>1,778</td>
<td>6,945</td>
<td>1,244</td>
<td>6,842</td>
<td>6,780</td>
<td>7,163</td>
<td>7,498</td>
<td>9,102</td>
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<tr>
<td></td>
<td>F</td>
<td>606</td>
<td>6,413</td>
<td>1,192</td>
<td>6,315</td>
<td>6,235</td>
<td>5,640</td>
<td>6,960</td>
<td>8,443</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Age</th>
<th>&lt; 20</th>
<th>21–30</th>
<th>31–40</th>
<th>41–50</th>
<th>&gt;50</th>
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<tbody>
<tr>
<td>sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>46</td>
<td>790</td>
<td>810</td>
<td>830</td>
<td>770</td>
</tr>
<tr>
<td>F</td>
<td>48</td>
<td>1,040</td>
<td>259</td>
<td>980</td>
<td>259</td>
</tr>
<tr>
<td>Se</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>46</td>
<td>79</td>
<td>810</td>
<td>83</td>
<td>770</td>
</tr>
<tr>
<td>F</td>
<td>48</td>
<td>73</td>
<td>259</td>
<td>85</td>
<td>259</td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>46</td>
<td>6,065</td>
<td>809</td>
<td>6,470</td>
<td>770</td>
</tr>
<tr>
<td>F</td>
<td>48</td>
<td>5,515</td>
<td>259</td>
<td>5,850</td>
<td>259</td>
</tr>
</tbody>
</table>

M = male, F = female, 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

<table>
<thead>
<tr>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>M</td>
<td>31</td>
<td>802</td>
<td>1,000</td>
<td>217</td>
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<tr>
<td></td>
<td>F</td>
<td>71</td>
<td>401</td>
<td>174</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>102</td>
<td>1,203</td>
<td>1,174</td>
<td>100</td>
</tr>
<tr>
<td>Se</td>
<td>M</td>
<td>29</td>
<td>82</td>
<td>999</td>
<td>218</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>71</td>
<td>79</td>
<td>175</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>100</td>
<td>80</td>
<td>1,174</td>
<td>278</td>
</tr>
<tr>
<td>Zn</td>
<td>M</td>
<td>31</td>
<td>6,480</td>
<td>1,000</td>
<td>217</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>71</td>
<td>5,950</td>
<td>173</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>102</td>
<td>6,165</td>
<td>1,173</td>
<td>277</td>
</tr>
</tbody>
</table>

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

![Fig. 2. Relationship between age and level of Cu in blood of men.](image1.png)

![Fig. 4. Relationship between BMI and level of Cu in blood of men.](image2.png)

![Fig. 3. Relationship between age and level of Cu in blood of women.](image3.png)

![Fig. 5. Relationship between age and level of Se in blood of Czech population.](image4.png)
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 concentrations 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 estrogen 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 presumtion 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

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![Fig. 6. Relationship between age and level of Zn in blood of women.](image1)

![Fig. 7. Relationship between BMI and level of Zn in blood of women.](image2)
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


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