Zinc, Copper, Manganese, and Selenium Status in Pre- and Postmenopausal Women During Sex Hormone Therapy

Stężenia cynku, miedzi, manganu i selenu u kobiet menopauzalnych przyjmujących terapię hormonalną

Abstract

Background. The impact of estrogen deficiency after menopause on trace minerals has not been widely studied.

Objectives. The aim of this study was to compare trace mineral levels of postmenopausal women with those of premenopausal women of similar age and to determine the impact of estrogen (ET) and estro-progestin (HT) therapy on them.

Material and Methods. E2, FSH, Cu, Zn, Se, and Mn concentrations in serum and/or whole blood in 80 postmenopausal women (M1 group: 26 with surgically induced and 54 with physiological menopause) and in 40 premenopausal controls (C) were determined. In the M1 group the measurements were repeated after 4 months of ET or HT.

Results. The study showed that trace mineral status in the postmenopausal women was slightly different from that of the C. The serum Zn level after menopause was considerably higher than that of the C and tended to decrease after ET, whereas it remained unchanged after HT. Serum Mn level after menopause was similar to that of the controls, but it decreased significantly after ET, whereas it remained unchanged after HT. The rest of the trace element concentrations showed only slight changes.


Key words: copper, zinc, selenium, manganese, menopause, hormonal therapy.

Streszczenie

Wprowadzenie. Wpływ niedoboru estrogenów na stężenia metali śladowych u kobiet po menopauzie nie został dotychczas dobrze poznany.

Cel pracy. Zbadano, czy stężenia Zn, Cu, Se i Mn w surowicy i krwi pełnej kobiet przed i po menopauzie różnią się oraz oceniono wpływ terapii estrogenowej (ET) i estroprogestagenowej (HT) na stężenia tych metali.

Materiał i metody. Porównano 80 zdrowych kobiet po menopauzie (54 – naturalnej, 26 – chirurgicznej) i 40 przed menopauzą (K). Oznaczano stężenia E2, FSH w surowicy oraz Zn, Cu, Se i Mn w surowicy i krwi pełnej, przed i po 4-miesięcznej terapii: u kobiet z zachowaną macicą – HT, u kobiet z menopauzą chirurgiczną – ET.

 Wyniki. Wykazano, że stężenia metali śladowych u kobiet po menopauzie nieznacznie różnią się od stężeń obserwowanych w grupie kontrolnej. Stężenie cynku w surowicy było większe u kobiet po menopauzie niż w grupie kontrolnej i pod wpływem terapii estroprogestagenowej (HT) nieznacznie zmniejszało się, pozostając bez zmian pod wpływem terapii estrogenowej (ET). Wyjaśnienie porównywalne między badanymi grupami stężenie manganu w surowicy istotnie zmniejszyło się pod wpływem ET i pozostało niezmienione pod wpływem HT. Stężenia pozostałych badanych mikroelementów zmieniały się jedynie w nieistotnym zakresie.


Słowa kluczowe: miedź, cynk, selen, mangan, menopauza, terapia hormonalna.
The decrease in sex steroid hormones during menopause in women causes a number of disturbances in the metabolisms of different organs. In this period of life, the risk of osteoporosis, cardiovascular diseases, arterial hypertension, impairment of glucose metabolism, breast cancer, and degenerative cognition diseases rises. The detailed mechanisms of this effect are still being investigated. The impact of estrogen deficiency after menopause on trace minerals has not yet been widely studied, but the expected menopause-related alterations in trace mineral status may have an impact on the above pathologies [1–4]. Several trace elements, particularly Ca, Mg, Cu, Mn, and Zn, are essential in bone metabolism [5, 6]. The authors previously examined the serum and whole-blood Ca and Mg concentrations in pre- and postmenopausal women before and after hormonal therapy, showing that menopausal women have significantly higher blood calcium levels than premenopausal women, which probably results from higher bone resorption of those cations. The authors observed that the blood Ca level decreased after four months of therapy with estrogens or estro-progestins, becoming comparable to that of premenopausal subjects. The Mg concentration showed a similar, but not statistically significant tendency to Ca concentration [7]. Some investigators have also shown that in postmenopausal women serum Zn level negatively correlates with serum glucose level, whereas in pre-menopausal ones it correlates positively. This may suggest that menopause-induced changes in Zn metabolism may have an impact on glucose tolerance [47]. Some trace minerals are cofactors of antioxidant enzymes. Se is a cofactor of glutathione peroxidase (GSH-Px), one of the most important enzymes of the free-radical defense system. Zn and Cu molecules are integrated elements of superoxide dismutase (Cu/Zn-SOD). Manganese superoxide dismutase (MnSOD) is a major enzyme responsible for the detoxification of reactive oxygen species in the mitochondria. The protective activities of the enzymes are dependent on the redox capability of the cations. The authors recently demonstrated that total antioxidant status (TAS) in untreated postmenopausal women is lower and after hormonal therapy it rises to a level comparable to that of premenopausal women [8]. It is thought that disturbances in trace mineral status during menopause may impair the antioxidant defense of postmenopausal women, enhancing the oxidative stress involved in most degenerative organ processes. It is documented that hormonal therapy has beneficial impact on the prooxidant-antioxidant balance in postmenopausal women [8–11]. Some investigators have shown that hormonal replacement therapy antagonizes the decrease in SOD activity that occurs after menopause [12]. It is also supposed that hormonal therapy may influence trace mineral status.

The aim of this study was to compare the trace mineral status of postmenopausal women with that of healthy, regularly cycling women of similar age and to determine the impact of estrogen and estro-progestin therapy on it.

### Material and Methods

#### Constitution of the Groups

One hundred and twenty healthy women were investigated. The study group consisted of 80 postmenopausal women (M1 group) who were divided into two subgroups: 26 women with surgically induced menopause (ET1 group) with a mean age of 50.9 ± 2.9 years and 54 women with physiological menopause (HT1 group) with a mean age of 50.5 ± 3.0 years. The control group (C) comprised 40 premenopausal healthy volunteers with a mean age of 48.3 ± 2.3 years. Every subject underwent physical and laboratory examination. Each of investigated women presented normal findings of blood cell count test, liver function test, serum glucose, urea and creatinine tests. None of the subjects were on a special diet, none were cigarette smokers, and none had used any medication during the four months proceeding the onset of the study. All the patients were informed about the aims and the methods of the study and gave their informed consent. The study protocol was approved by the Bioethics Committee of the Wroclaw Medical University, Poland.

All the postmenopausal women were treated for four months (M2 – the postmenopausal group after treatment). The ET1 group received estradiol (E2) transdermally (estrogen therapy – ET) in a dose of 50 μg daily (the ET1 group after treatment was designated as the group ET2). The HT1 group received combined hormonal therapy (HT) consisting of E2 given transdermally in a dose of 50 μg daily and medroxyprogesterone acetate (MPA) administered in sequential fashion in a dose of 5 mg daily for 12 days in the second phase of the cycle (the HT1 group after treatment was designated as the group HT2).

#### Sample Collection

Blood samples were collected by vein puncture between 8:00 and 10:00 a.m., after overnight fasting, and were stored at −30°C.
Trace Mineral and Hormone Analyses

Serum E2 concentration was determined by radioimmunoassay (RIA, Diagnostic Products Corporation, Los Angeles, CA, USA) and serum FSH concentration was determined by immunoradiometric assay (IRMA, Biodata Diagnostics, Rome, Italy).

Blood samples for Se measurement were microwave digested with nitric acid in a self-contained system. A Plazmatronika-Servis BM-11 Microwave Digestion Unit with high-density Teflon vessels was used. The digestion of samples was followed by a reduction step with concentrated hydrochloric acid to convert Se(VI) to Se(IV). Se levels were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) on an Arl 3410 ICP vacuum spectrometer using the 196.094 nm spectral line with hydride generation (the online Arl hydride generator produced by ROTH, Karlsruhe, Germany, and 1.43% potassium borohydride solution were used). The calibration curves were obtained using blood, a selenium reference solution (Aldrich), and the Merck 9976 Titrisol reference solution. Nycomed reference materials were controls.

Cu, Zn, and Mn concentrations in serum and whole blood were determined by atomic absorption spectrophotometry on a SOLAAR M6 (Thermo Elemental). For Cu and Zn measurements an air-acetylene flame was used at the 324.8 nm wavelength for Cu and 213.9 nm for Zn with deuterium background correction. The reference material PEAK PERFORMANCE certified by ICP-NIST SRM 3114 was used. For the Mn measurement an electrographite cuvette was used at the wavelength of 279.5 nm with Zeeman’s background correction. Seronorm Whole Blood 3 SPEX Certiprep 2 (Dalston Gardens Stanmore Middlesex HA7 1BQ, UK) was used as the reference material.

Statistical Analysis

Statistical analysis was performed using Student’s t-test; p-values < 0.05 were considered statistically significant. Correlation analysis was performed by calculating Pearson’s or Spearman’s correlation coefficients. Results were considered statistically significant with $p < 0.05$.

Results

The postmenopausal women had a significantly lower mean serum E2 level and higher FSH level than the C group. After HT and ET, serum E2 rose significantly and serum FSH fell significantly in the postmenopausal groups (Table 1).

The whole-blood ($Zn_w$, $Cu_w$, $Se_w$) and serum ($Zn_s$, $Cu_s$, $Se_s$, $Mn_s$) trace metal concentrations in postmenopausal women before and during substitution therapy and in the control group are presented in Table 2. The whole-blood Zn, Se, and Cu concentrations were not statistically different between the groups M1, M2, and C. Zn serum level was significantly higher in group M1 than in C and did not change after sex hormone therapy. In the ET1 and HT1 groups the serum level of Zn was significantly higher than in the C group. After ET the serum Zn concentration tended to decrease, but the difference was not statistically significant, whereas in the group treated with estro-progestin (HT), the serum Zn concentration remained unchanged after therapy. The whole-blood Zn concentration in M1 was not significantly higher than in C and after administration of both types of therapy it tended to decrease slightly, becoming similar to C. Whole-blood and serum Se concentrations in the M1 and M2 groups did not differ significantly from those of the group of healthy controls. It rose slightly in whole blood, whereas it rather tended to decrease in plasma after both types of therapy, but all the differences were not statistically significant. Whole-blood and serum Cu concentrations in M1 were not significantly lower than in C and showed a tendency to rise after both types of therapy, becoming comparable to those of C. The serum Mn concentrations in the M1 and M2 groups were not significantly different from that of the controls (C). It decreased after estrogen therapy, achieving a significantly lower level in the ET2 group than before therapy (in ET1) and in the premenopausal controls (C), whereas in the group treated with estro-progestin (HT), the serum Mn concentration remained unchanged after therapy.

There were no statistically significant correlations in each group of women between the concentrations of the studied trace minerals in the serum or whole blood and serum hormone concentrations before and after both types of sex hormone therapy. There were also no correlations observed between the trace metal levels and clinical features, such as BMI or systolic and diastolic blood pressure.

Discussion

The present study shows that trace mineral status in postmenopausal women is only slightly different from that of premenopausal women of similar age. The authors observed that postmenopausal women have higher concentrations...
of Zn in serum and whole blood than premenopausal, but the difference is significant only for the serum Zn level. Similarly, a previous study of a Czech population reported that blood Zn concentration increased with age in women [13]. In another study, higher urinary Zn loss in postmenopausal non-HT women was observed which was also associated with higher Ca and Mg urinary loss [14]. Own study shows that hormonal therapy does not significantly alter elevated serum and whole-blood Zn concentrations in postmenopausal women, but a slight tendency to decrease is observed. It is suggested that higher Zn concentration in postmenopausal women, which is also involved in bone metabolism, might be an effect of the increased bone resorption that appears with estrogen deficiency. Studies by some investigators have demonstrated a decrease in plasma Zn concentration accompanied, in some cases, by a reduction in urinary Zn loss, both induced by the administration of estrogens to postmenopausal women [14–18]. An indirect confirmation of these data is also a decrease in Zn serum level reported in a study of healthy pregnant women [19]. Own study is compatible and confirms the already existing observations. The decrease in Zn level may be partially due to a decrease in circulating albumins [14, 15], but intestinal Zn absorption or increased bone Zn buildup could also be affected. In experimental research on rats it was shown that intestinal absorption of Zn considerably decreased with age. It is supposed that similar changes may play

<table>
<thead>
<tr>
<th>Group (Grupa)</th>
<th>Weight (Masa ciała) (kg)</th>
<th>BMI (kg/m²)</th>
<th>WHR</th>
<th>RR_{semen} (mm Hg)</th>
<th>RR_{drams} (mm Hg)</th>
<th>Age – years (Wiek – lata)</th>
<th>FSH (miU/ml)</th>
<th>E₂ (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M₁ (n = 80)</td>
<td>x</td>
<td>± 12.0</td>
<td>0.78</td>
<td>± 0.47</td>
<td>138.1&lt;sup&gt;d&lt;/sup&gt; ± 18.4</td>
<td>88.3&lt;sup&gt;d&lt;/sup&gt; ± 11.7</td>
<td>59.0&lt;sup&gt;a&lt;/sup&gt; ± 2.9</td>
<td>82.1&lt;sup&gt;d&lt;/sup&gt; ± 36.9</td>
</tr>
<tr>
<td>M₂ (n = 80)</td>
<td>x</td>
<td>± 11.6</td>
<td>0.77</td>
<td>± 0.42</td>
<td>132.9&lt;sup&gt;d&lt;/sup&gt; ± 16.7</td>
<td>85.2&lt;sup&gt;d&lt;/sup&gt; ± 9.5</td>
<td>59.0 ± 2.9</td>
<td>45.8&lt;sup&gt;h&lt;/sup&gt; ± 26.5</td>
</tr>
<tr>
<td>ET₁ (n = 26)</td>
<td>x</td>
<td>± 12.6</td>
<td>0.79</td>
<td>± 0.05</td>
<td>143.9&lt;sup&gt;b,c&lt;/sup&gt; ± 20.7</td>
<td>94.1&lt;sup&gt;b,c&lt;/sup&gt; ± 10.6</td>
<td>51.6 ± 2.6</td>
<td>75.7&lt;sup&gt;b&lt;/sup&gt; ± 37.5</td>
</tr>
<tr>
<td>ET₂ (n = 26)</td>
<td>x</td>
<td>± 12.4</td>
<td>0.78</td>
<td>± 0.04</td>
<td>136.0&lt;sup&gt;b&lt;/sup&gt; ± 18.5</td>
<td>84.8&lt;sup&gt;b&lt;/sup&gt; ± 10.4</td>
<td>51.6 ± 2.6</td>
<td>52.5&lt;sup&gt;b&lt;/sup&gt; ± 21.1</td>
</tr>
<tr>
<td>HT₁ (n = 54)</td>
<td>x</td>
<td>± 11.5</td>
<td>0.78</td>
<td>± 0.04</td>
<td>131.5&lt;sup&gt;e&lt;/sup&gt; ± 15.4</td>
<td>83.3&lt;sup&gt;e&lt;/sup&gt; ± 10.0</td>
<td>50.5&lt;sup&gt;e&lt;/sup&gt; ± 3.0</td>
<td>87.0&lt;sup&gt;e&lt;/sup&gt; ± 36.3</td>
</tr>
<tr>
<td>HT₂ (n = 54)</td>
<td>x</td>
<td>± 10.9</td>
<td>0.77</td>
<td>± 0.04</td>
<td>131.1&lt;sup&gt;e&lt;/sup&gt; ± 15.4</td>
<td>83.2&lt;sup&gt;e&lt;/sup&gt; ± 8.4</td>
<td>50.5 ± 3.0</td>
<td>40.7&lt;sup&gt;k&lt;/sup&gt; ± 29.3</td>
</tr>
<tr>
<td>C (n = 40)</td>
<td>x</td>
<td>± 8.6</td>
<td>0.78</td>
<td>± 0.04</td>
<td>130.3&lt;sup&gt;b&lt;/sup&gt; ± 14.7</td>
<td>82.2&lt;sup&gt;b&lt;/sup&gt; ± 9.1</td>
<td>48.3&lt;sup&gt;b&lt;/sup&gt; ± 2.3</td>
<td>7.70&lt;sup&gt;b&lt;/sup&gt; ± 4.56</td>
</tr>
</tbody>
</table>

M₁ – postmenopausal group before treatment. M₂ – postmenopausal group after treatment. ET₁ – group with surgical menopause before ET. ET₂ – group with surgical menopause after ET. HT₁ – group with natural menopause before HT. HT₂ – group with natural menopause after HT. C – control group. x – average. SD – standard deviation. Statistically significant differences between groups (p < 0.05):


Statistically significant differences between groups (p < 0.05):


Table 1. Clinical features and sex hormones concentrations of the groups

Tabela 1. Charakterystyka kliniczna i stężenia hormonów płciowych w badanych grupach
Menopause and Zn, Cu, Mn and Se

A role in the human trace element balance in later life [20]. Many studies of menopausal women reported higher Ca and Mg serum concentrations than in healthy cycling women which were associated with higher urinary loss of these ions. The administration of HT has been reported to reverse these changes [7, 14, 21–24]. There is an analogy between Ca and Mg concentration changes and postmenopausal fluctuations of Zn concentration. The reversion of changes in Mg and Ca, as well as Zn, blood concentration and urinary excretion after HT may be partially ascribed to an estrogen-induced increase in the bone buildup process, which is crucial for preventing osteoporosis.

In own study, whole-blood and serum Se concentrations in the M1 and M2 groups did not differ statistically significantly from those of the group of the healthy cycling controls. Serum Se concentration showed only a slight tendency to be higher than in controls, whereas the whole-blood Se level appeared to be lower than in the healthy cycling women. These data on the serum Se level correspond with other authors’ observations. Smith et al., in a cross-sectional study of three generations of women in America, observed that the serum Se concentration in women aged 40–58 years was significantly higher than that of their daughters and mothers [25]. Another study of a Portuguese
population showed an age-dependent increase in serum Se level, regardless of sex [26]; the same was found in a study of a Czech population [13]. The slightly, but not statistically significantly, higher serum Se concentration in the group of postmenopausal women in our study, who were a little bit older than the controls, agrees with the above data. Own study showed that HT did not significantly alter Se levels. It increased slightly in whole blood, whereas it rather decreased in plasma after both types of therapy. Ha et al., who observed Se fluctuation during the menstrual cycle in healthy young women, had different findings. They noted that during the estrogen peak in the periovulatory phase the serum Se concentration as well as serum and erythrocyte GPx activities were greatest compared with the other phases of the ovulatory cycle [27]. In own previous study on antioxidant defense in postmenopausal women the authors also found a simultaneous increase in erythrocyte GSH-Px and whole-blood Se concentration (indicating the Se erythrocyte level) during HT [8].

Ha et al. found in their investigation a positive relationship between plasma estrogen and Se; a similar relationship was found by Smith et al. in a cross-sectional study of American daughters, mothers, and grandmothers [25, 27]. Own data did not confirm such a relationship.

The whole-blood and serum Cu concentrations in our study were slightly, but not statistically significantly, lower than in the controls, which agrees with previous reports by other authors that the Cu concentration decreases with age in women [13]. The authors showed that the administration of estrogens and/or estroprogestins during hormonal replacement therapy caused a tendency to increase Cu concentration to the level of the controls. These data are similar to those of the other investigators. In the study by Bureau and colleagues, hormone therapy in postmenopausal women caused a significant increase in serum copper level, while it remained unmodified by treatment in erythrocytes [14]. The recent study of a Portuguese population reported that hormonal replacement therapy does not significantly affect serum Cu concentration [26]. Other studies show that the administration of sex hormones in oral contraceptives is connected with an increase in serum copper concentration [13, 28]; similarly, it has been shown in Spanish and Polish populations that serum Cu level increases progressively during pregnancy, when estrogen levels are higher than in non-pregnant women [19, 29]. It has also been experimentally shown that estrogen deficiency leads to a decrease in copper content in rat teeth and mandible, and giving 17 beta-estradiol positively influences the content of mineral components in these tissues [30]. The impact on Cu concentration has been ascribed to an estrogen-induced release of ceruloplasmin in the liver [28, 31]. An adequate copper status plays a crucial role in osteoporosis [30, 32]; however, the fact that in cohort studies high serum Cu levels have been associated with increased cardiovascular mortality [33–35], heart failure [36], and cardiac markers in acute coronary syndromes [37] cannot be neglected in the discussion. Estrogen and hormonal therapy might have some influence on increasing Cu concentration, which may be considered negative for the cardiovascular system, but the crucial influence of estrogens on the cardiovascular system is beneficial and depends not on trace element fluctuations, but on many positive changes in lipid profile, the functioning and structure of the vascular wall, inflammatory processes, and the prooxidant-antioxidant balance [38, 39].

In own study the serum manganese concentration in the postmenopausal group was not significantly different from that of the group of healthy cycling controls. However, the authors observed that estrogen therapy significantly decreased serum Mn concentration, whereas treatment with estroprogestins did not change it. This result is in agreement with Bureau's observation that combined hormonal therapy does not change serum Mn level in postmenopausal woman [14]. There is still little evidence in the literature about the influence of estrogen on serum Mn concentration in humans. Elevated manganese levels have been reported in congestive heart failure, infections, and psychoses [4]. Interaction between manganese and estrogens has recently been shown by Rahnema et al. in an experimental study on female rats. After ovariectomy, the level of manganese in the teeth as well as in the mandible decreased significantly compared with a control group, and the administration of 17 beta-estradiol caused an considerable increase in manganese content [40]. These results confirm that ovarian estrogens play an important role in the mineralization of bones and teeth. Own findings are consistent with experimental data and may therefore be explained by an estrogen-induced increase in the bone buildup process, which is crucial for preventing osteoporosis and tooth demineralization. Mn is a cofactor of an important mitochondrial antioxidative enzyme, MnSOD. There is some evidence that a genetic polymorphism in MnSOD may be associated with an increased risk of breast cancer [41] and the development of diabetic nephropathy [42], but in the large-population Nurses’ Health Study there was no relationship found between MnSOD genotype and postmenopausal hormone use [43]. An interesting fact is that it has been recently observed...
that higher hair Mn concentration resulting from a high intake of Mn in drinking water correlated positively with hyperactive behaviors, cognitive problems/inattention, and higher ADHD index among Canadian children [44]. Does the higher serum Mn concentration among postmenopausal women play any role in the universal menopausal syndrome, particularly consisting of concentration difficulties and emotional hyper-reactivity? The hypothesis needs further investigation.

Trace element status in different populations of women from different countries depends largely on the geographic area of residence, diet, and the general environmental and socioeconomic conditions. For example, it has recently been reported that 10% of the Algerian population had plasma zinc levels lower than 10.6 µmol/l, whereas copper and selenium status seemed to be satisfactory [45]. In New Zealand, where the soil is poor in Se, 80% of the female population of nonusers of selenium supplements had plasma selenium levels approximately 10% below the mean plasma selenium level necessary for full expression of GSH-Px activity and lower serum zinc levels [46].

The authors concluded that the estrogen deficiency after menopause may be the cause of some small fluctuations in the serum and blood concentrations of trace minerals. The precise impact of this deficiency and the influence of estrogen or estroprogestin therapy on trace mineral status in postmenopausal women needs further larger-population studies.

References


Address for correspondence:
Anna Jodkowska
Department of Internal Medicine, Occupational Diseases and Hypertension
Wroclaw Medical University
Pasteura 4
50-367 Wrocław
Poland
Tel.: +48 601 359 139
E-mail: agladzia@poczta.onet.pl

Conflict of interest: None declared

Received: 8.03.2010
Revised: 9.04.2010
Accepted: 7.06.2010