ZINC DEFICIENCY AND SUPPLEMENTATION IN OVARIECTOMIZED RATS: THEIR EFFECT ON SERUM ESTROGEN AND PROGESTERONE LEVELS AND THEIR RELATION TO CALCIUM AND PHOSPHORUS

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ABSTRACT
The aim of this study is to examine how zinc deficiency or supplementation affects estrogen and progesterone and calcium and phosphorus levels in the serum. The study was carried out on 40 adult female rats of Spraque-Dawley species. The rats were allocated to four groups: Group 1: Control, Group 2: Ovariectomized (OVX) control. Group 3: OVX-Zinc-supplemented. Group 4: OVX-Zinc-deficient. Blood samples were taken from the experimental animals by decapitation method and analyzed in terms of estrogen, progesterone, calcium, phosphorus, magnesium and zinc levels.

Group 1 had the highest estrogen levels (p<0.05). Estrogen levels in group 3 were higher than those in groups 2 and 4 (p<0.05). The lowest estrogen levels were found in group 4 (p<0.05). Progesterone levels were higher in group 1 than in groups 2, 3 and 4 and the same parameter in group 3 was higher than those in groups 2 and 4. The highest calcium and phosphorus levels were obtained in groups 1 and 3 (p<0.05). Calcium and phosphorus levels in group 2 were higher than those in group 4 (p<0.05). There was no difference among groups with regard to magnesium levels. Group 3 had the highest serum zinc levels (p<0.05). Zinc levels in group 1 were higher than those in groups 2 and 4 and the levels in group 2 were higher than those in group 4.

Findings of the study show that zinc deficiency causes a significant decrease in calcium and phosphorus levels and that zinc supplementation prevents these adversities in ovariectomized rats.

Keywords: Ovariectomy, zinc, ovary hormones, calcium and phosphorus.

INTRODUCTION
Zinc, which is known to have extensive and crucial roles in the mammal system, is regarded a key trace element for the growth of humans and many animal species (Prasad, 1985; Vallee and Falchuk, 1993). There is a vital relation between zinc and bone growth. Zinc stimulates reproduction and differentiation in osteoblastic cells and inhibits osteoclastic activity in the bone tissue. Consequently, zinc encourages protein synthesis in osteoblastic cells and plays a part in the preservation of bone mass (Igarashi and Yamaguchi, 1999). It was demonstrated that zinc functioned as a co-factor for specific enzymes in the bone metabolism and that zinc supplementation increased spinal bone mineral density in menopausal women (Saltman and Strause, 1993). It has been noted in a study including menopausal women that urinary zinc discharge increased in menopausal period and that there was an important relation between osteoporosis and zinc and one between zinc and calcium (Contreras et al., 2002). It was concluded in the same study that determination of zinc levels could be a useful criterion in the diagnosis of osteoporosis (Contreras et al., 2002). It was observed in a rat study where diabetes was induced by streptozotocin and a zinc-deficient diet was administered that zinc deficiency significantly increased calcium-phosphorus discharge when compared to diabetic rats fed on a normal diet and thus there was more bone damage in zinc-deficient diabetic rats than in diabetic controls. Interestingly, bone damage observed in diabetic control animals could be restored by insulin administration, whereas the damage in diabetic rats fed on a zinc-deficient diet could not be mended despite insulin administration (Fushimi et al., 1993). This impressive piece of information is a crucial finding indicating that zinc can have a significant impact on bone metabolism. It has been found that calcium supplementation at the same rate with zinc supplementation could better prevent the losses in bone tissue (Strause et al., 1994) and it is claimed that estrogen inadequacy in post-menopausal women increased zinc discharge (Szatmhari et al., 1993), while estrogen replacement prevented it (Herzberg et al., 1996). The anabolic effect brought about by zinc supplementation in metaphysical tissues in culture setting can be presented as further proof of the relation between zinc and bone metabolism (Yamaguchi and Gao, 1998). It was shown that AHZ (beta-alanyl-L-histidinato zinc) administration to ovariectomized rats restored disorders in bone metabolism (Segawa et al., 1993) and increased zinc accumulation in femoral diaphysis (Yamaguchi and Kishi, 1993), as well as trabecular formation in femoral metaphysis (Kishi et al., 1994). Consequently, one can say that there is an important relation between zinc and changes in bone metabolism. The aim of the present study

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is to investigate how zinc deficiency and zinc supplementation affects estrogen, progesterone, calcium and phosphorus levels in the serum in ovariectomized rats.

MATERIALS AND METHODS

The study was conducted in the Experimental Medicine Research and Application Center of Selcuk University (SUDAM) on rats provided by the Center. The ethics committee of SUDAM approved the study protocol. The study included 40 adult female rats of Spraque-Dawley species, which were allocated to four groups as follows:

Group 1 (n=10): the control group that was not subjected to any procedure.
Group 2 (n=10): the group fed on a normal diet after being ovariectomized under general anesthesia.
Group 3 (n=10): the group supplemented with intraperitoneal zinc (3 mg/kg/day) for six weeks after being ovariectomized under general anesthesia.
Group 4 (n=10): the group fed on a zinc-deficient diet (0.65 ppm zinc/g diet) for six weeks after being ovariectomized under general anesthesia.

In order to minimize zinc contamination, the experimental animals were fed in special steel cages which were cleaned daily by washing. The feed were given in special steel bowls and water in glass feeding bottles. Both zinc-deficient and normal forms of the animal feed were prepared in Korkuteli Feed Supplement Industry Factory (in Korkuteli).

Zinc sulfate administration

After being dissolved in distilled water, zinc sulfate was administered in 0.5 ml serum physiological in the form of 3 mg/kg/day intraperitoneal injections. Zinc sulfate injections were made at the same hour of the day (at 09.00 a.m.) for six weeks.

Surgical procedures

The rats in groups 2, 3 and 4 were ovariectomized under general anesthesia (60 mg/kg ketamine and 5 mg/kg rompun). The hair on the back of the rats was shaved. Following appropriate asepsis and antisepsis with betadine, rats were placed into ventral position. The skin was incised from the 1/3 upper part of the distance between median section of the back and tail. After subcutaneous tissues were freed, spinal muscle was reached. Peritoneal cavity was accessed through abdomen’s back wall muscles. Ovaries were taken out together with the fat tissue. Ovaries were cleared of the fat tissue, clamped, ligated and cut. After bleeding control, other organs were put back into the peritoneal cavity. The muscle was sutured with 2/0 chrome catgut and skin with 2/0 silk (Waynfort and Flecnell, 1994).

Serum estrogen and progesterone analyses

In order to analyze estrogen and progesterone in the serum, blood samples were collected from all the experimental animals by decapitation method. After clotting, the samples were centrifuged at 3000 rpm to separate serum. Hormone analyses were carried out in the Main Biochemistry Laboratory of Selcuk University Meram School of Medicine using IMMULITE 2000 equipment. Estrogen levels were determined using catalogue no: L2KE22 kit as pg/ml and progesterone levels were determined using catalogue no: L2KPW2 kit as ng/ml.

Serum calcium, phosphate and magnesium analyses

Calcium, phosphate and magnesium analyses in the serum samples of the rats were conducted in the Main Biochemistry Laboratory of Selcuk University Meram School of Medicine using Olympus AU 2700 autoanalyzer. Serum potassium was analyzed by ion selective method, while calorimetric method was employed in other analyses. Calcium, phosphate and magnesium levels were presented as mg/dl.

Serum zinc analyses

Serum zinc was analyzed in Shimatsu ASC-600 Atomic Absorption Spectrophotometer in the Biochemistry Department of Elazığ Firat University, Medical School. The measurements were repeated twice for each sample using flame atomization technique with light at 213.9 wavelength. Zinc levels were expressed as µg/dl.

STATISTICAL ANALYSES

Statistical evaluation of the findings was made using Minitab for Windows Release 13.0 computer software. Arithmetic mean values and standard errors of all parameters were calculated. Variance analysis was employed to determine the differences among groups. Level of significance was set at p<0.01.

RESULTS

When the groups were compared in terms of mean body weights, it was seen that there was no difference among them at the beginning of the study. However, at the end of the six-week study, mean weight in the ovariectomized group fed on a zinc-deficient diet (group 4) was found significantly lower than those in groups 1, 2 and 3 (p<0.05, table 1).

Of the parameters presented in table 2, estrogen levels were the highest in the control group which was not subjected to any procedure (group 1) (p<0.05). This parameter was higher in the ovariectomized and zinc-
supplemented group (group 3) than it was in groups 2 and 4 (p<0.05). Estrogen levels in the ovariectomized control group (group 2) were higher than those in group 4 and lower than those in groups 1 and 3 (p<0.05). Estrogen levels in the zinc-deficient, ovariectomized group (group 4) were found significantly lower than those in the remaining groups (p<0.05).

Calcium and phosphorus levels in groups 1 and 3 were not different from each other, but were higher than those levels in groups 2 and 4 (p<0.05). Group 4 had the lowest serum calcium and phosphorus levels. Serum magnesium levels were not significantly different in any group. Serum zinc levels in the zinc-supplemented and ovariectomized group (group 3) were found higher than those in the rest of the groups (p<0.05). The control group, which was not subjected to any procedure, had serum zinc levels lower than those in group 3 (p<0.05), but higher than those in groups 2 and 4 (p<0.05). Serum zinc levels were higher in the ovariectomized control group (group 2) than in group 4 (p<0.05). Serum zinc levels in the zinc-deficient ovariectomized group were significantly lower than those in the remaining groups (p<0.05, table 3).

**DISCUSSION**

Although mean weights of the groups at the beginning of the study were not different, it has been reported that zinc-deficient group 4 had a significant weight loss at the end of the study. It can be said that the weight loss observed in group 4 was an expected result, as it was demonstrated in several studies that zinc deficiency in the diet caused weight loss (Fraker et al., 1982). Besides, it is a widely accepted view that the most obvious indicator of zinc deficiency is inadequate food intake, in other words loss of appetite, and a decrease in body weight (Safai-Kutti and Kutti, 1986). The weight loss we observed in the zinc-deficient group is consistent with the reports to the effect that zinc deficiency in animals led to anorexia, weight loss, poor food efficiency and delays in growth.

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**Table 1**: Weight changes in the study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight before the study (g)</th>
<th>Weight after the study (g)</th>
<th>Weight change (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Normal Control</td>
<td>202.50±10.34</td>
<td>225.00±13.98</td>
<td>23.50±16.74</td>
</tr>
<tr>
<td>2 O VX-Control</td>
<td>201.50±15.99</td>
<td>226.50±23.92</td>
<td>25.00±31.82</td>
</tr>
<tr>
<td>3 O VX-Zinc-supplemented</td>
<td>202.00±9.19</td>
<td>223.50±13.95</td>
<td>21.00±16.63</td>
</tr>
<tr>
<td>4 O VX-Zinc-deficient</td>
<td>202.50±15.14</td>
<td>188.50±12.03</td>
<td>-13.50±10.55</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Mean values with different superscripted letters in the same column have statistical significance (P<0.05)

**Table 2**: Serum estrogen and progesterone levels in the study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Estrogen (pg/ml)</th>
<th>Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Normal Control</td>
<td>62.21±4.46</td>
<td>18.08±1.20</td>
</tr>
<tr>
<td>2 O VX-Control</td>
<td>10.90±1.43</td>
<td>1.92±0.97</td>
</tr>
<tr>
<td>3 O VX-Zinc-supplemented</td>
<td>19.01±2.50</td>
<td>5.27±0.98</td>
</tr>
<tr>
<td>4 O VX-Zinc-deficient</td>
<td>5.68±1.42</td>
<td>1.11±0.57</td>
</tr>
<tr>
<td>P</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Mean values with different superscripted letters in the same column have statistical significance (P<0.05)

**Table 3**: Serum calcium, phosphorus, magnesium and zinc levels in the study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Calcium (mg/dl)</th>
<th>Phosphorus (mg/dl)</th>
<th>Magnesium (mg/dl)</th>
<th>Zinc (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Normal Control</td>
<td>10.43±0.39</td>
<td>4.74±0.55</td>
<td>2.36±0.11</td>
<td>91.30±6.52</td>
</tr>
<tr>
<td>2 O VX-Control</td>
<td>8.12±0.42</td>
<td>3.47±0.66</td>
<td>2.34±0.15</td>
<td>80.60±7.69</td>
</tr>
<tr>
<td>3 O VX-Zinc-supplemented</td>
<td>10.95±0.41</td>
<td>4.73±0.65</td>
<td>2.36±0.21</td>
<td>129.20±11.50</td>
</tr>
<tr>
<td>4 O VX-Zinc-deficient</td>
<td>7.23±0.48</td>
<td>2.50±0.47</td>
<td>2.32±0.17</td>
<td>50.60±6.40</td>
</tr>
<tr>
<td>P</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

*Mean values with different superscripted letters in the same column have statistical significance (P<0.05)
As expected, estrogen and progesterone levels in the control group (group 1) were higher than those in all the ovariec-tomized groups. When ovariec-tomized groups were compared that zinc supplemented ovariec-tomized group (group 3) had significantly higher estrogen and progesterone levels than both the ovariec-tomized control group (group 2) and the zinc-deficient ovariec-tomized group (group 4). The ovariec-tomized group fed on a zinc-deficient diet (group 4) had the lowest serum estrogen levels. Zinc has a key role in the physiology of the reproductive system (Hafiez et al., 1990). It is known that zinc deficiency in the diet in particular leads to hypogonadism (Nishi, 1996). However, it is seen that previous studies on the topic generally concentrate on the male reproductive system (Hafiez et al., 1990; Nishi, 1996; Prasad, 1985). Om and Chun (1996) showed that zinc deficiency led to an inhibition in LH and estrogen levels. An impressive example of the relation between zinc and female reproductive system is that estradiol and progesterone receptors obtained from calf uterus were bound to iminodiacetate-sepharose chelate colons that contained zinc (Vallee and Falchuk, 1993). In the present study, we found increased estrogen levels despite ovariec-tomy in the ovariec-tomized and zinc-supplemented rats (group 3) and further decreased estrogen levels in the ovariec-tomized and zinc-deficient group (group 4) in comparison to the ovariec-tomized control group; these findings point out a crucial relation between zinc and estrogen. It was argued that estrogen replacement after menopause reduced zinc discharge from the kidneys and thus zinc could be used as a new marker of changes in the bone metabolism (Herzberg et al., 1996). Parallel to this argument, Szatmahari et al. (1993), who noted decreased estrogen levels and increased zinc discharge in osteoporosis in menopausal women, suggested that zinc could be a significant criterion in determining changes in the bone metabolism. However, studies of both researchers address the effect of estrogen on zinc. Our study, on the other hand, focuses on the effects of zinc on estrogen. Besides, when the findings of the above-mentioned researchers are evaluated together with the increased estrogen levels we obtained with zinc supplementation or the decreased estrogen levels we observed in zinc deficiency, it can be said that there is a two-way relation between zinc and estrogen. Besides, the fact that zinc supplementation increases estrogen levels and zinc deficiency lowers the same parameter in ovariec-tomized rats suggests that zinc can affect estrogen secretion from suprarenal glands. We did not use hormone treatment in the menopausal rats in our study. Our aim was to establish a possible relation between zinc-deficient diet and zinc supplementation, and calcium and bone metabolism by eliminating other factors in ovariec-tomized rats. Actually, Humeny et al. (1999) reporting that zinc was a significant stimulator in estradiol synthesis is a remarkable finding supporting the relation we found between zinc and estrogen.

In this study we obtained the highest serum calcium and phosphorus levels in the control group (group 1) and the zinc-supplemented ovariec-tomized group (group 3). The lowest serum calcium and phosphorus levels, on the other hand, were found in the ovariec-tomized group fed on a zinc-deficient diet (group 4). It has been reported that there was a decline in calcium and phosphorus levels and an increase in urinary calcium and phosphorus discharge in diabetic rats with induced zinc-deficiency (Fushimi et al., 1993). Additionally, O’Dell et al. (1997) stated that zinc deficiency resulted in an inadequacy of calcium absorption. It was noted that levels of 1,25-dihydroxycholecalciferol levels decreased in zinc deficiency and that zinc stimulated 1,25-dihydroxycholecalciferol synthesis (Kimmel et al., 1991). Increased calcium and phosphorus levels in zinc supplementation and decreased levels in zinc deficiency we obtained in this study are parallel to literature data. We could not find a significant difference between magnesium levels of the groups. This finding indicates that when compared to calcium and phosphate levels in the serum, serum magnesium did not change with six-week zinc deficiency and/or zinc supplementation.

In the present study, the highest serum zinc levels were obtained in the zinc-supplemented ovariec-tomized group (group 3). Similarly, the ovariec-tomized group that was fed on a zinc-deficient diet (group 4) had the lowest serum zinc levels. The serum zinc levels in the ovariec-tomized control group (group 2) which was not subjected to any dietary application were significantly lower than those in the control group which was not subjected to any procedure (group 1). It is recognized that ovarian hormone deficiency in menopause stimulates bone loss. Ovariec-tomy can lead to osteoporosis by causing lack of estrogen (Yamaguchi and Kishi, 1993). Szatmahari et. al. (Szatmahari et al., 1993) demonstrated that urinary zinc discharge increased in postmenopausal women and thus estrogen insufficiency also led to zinc deficiency. It was shown in a study including 140 postmenopausal women that urinary zinc discharge increased in the absence of estrogen when compared to the controls and that there was a considerable relation between osteoporosis and zinc discharge (Herzberg et al., 1996).
In conclusion:
1. Six-week ovariectomy results in a significant decrease in serum zinc levels in rats.
2. When compared to ovariectomized rats fed on a normal diet, ovariectomized rats fed on a zinc-deficient diet have a significant inhibition in serum estrogen levels as well as a considerable decline in calcium and phosphorus levels.
3. Zinc supplementation helps to maintain serum calcium and phosphorus levels at the normal level in ovariectomized rats.
4. Zinc supplementation to ovariectomized rats can be useful in restoring the calcium mechanism.

REFERENCES


