Zinc Deficiency And Supplementation In Rats: Their Relation To Calcium And Phosphorus

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Abstract
Aim of the study: To examine how zinc deficiency or supplementation affects calcium and phosphorus levels in the serum.

Methodology: This study was carried out on 30 adult rats of Spraque-Dawley species. The rats were allocated to three groups: Group 1: Control, Group 2: Zinc-supplemented, Group 3: Zinc-deficient. Blood samples were taken from the experimental animals and analysis was done in terms of Calcium, Phosphorus, Magnesium and Zinc levels.

Result: The highest calcium and phosphorus levels were obtained in groups 1 and 2 (p<0.05). Calcium and phosphorus levels were lower in group 3. There was no difference among groups with regard to magnesium levels. Group 2 had the highest serum zinc levels (p<0.05). Zinc levels in group 1 were higher than those in groups 3.

Conclusion: 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 menopausal women.

Keywords: Zinc, Calcium and Phosphorus.

I. Introduction
Zinc is one of the most abundant nutritionally essential trace elements in the human body and is known to be necessary for normal bone metabolism (1). It is found in all body tissues with 85% of the whole body zinc in muscle and bone, 11% in the skin and the liver and the remaining in all the other tissues (2). The participation of trace minerals in normal development and maintenance of the skeleton is related to their catalytic functions in organic bone matrix synthesis (3). Zinc (Zn) regulates secretion of calcitonin from thyroid gland and influences bone turnover (4). Zn deficiency causes reduction in osteoblastic activity, collagen and chondroitin sulfate synthesis and alkaline phosphatase activity (5).

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 (6). There is a vital relation between zinc and bone growth, spermatogenesis and embryonic development (18). 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 (7). 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 (8).

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 (9). It was concluded in the same study that determination of zinc levels could be a useful criterion in the diagnosis of osteoporosis (9).

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 (10). 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 (8) and it as claimed that estrogen inadequacy in post-menopausal women increased zinc discharge (11), while estrogen replacement prevented it (12). 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 (13).

It was shown that AHZ (beta-alanyl-L-histidinato zinc) administration to ovariectomized rats restored disorders in bone metabolism and increased zinc accumulation in femoral diaphysis (14), as well as trabecular formation in femoral metaphysis (15).
Consequently, one can say that there is an important relation between zinc and changes in bone metabolism. The aim of the present study is to investigate how zinc deficiency and zinc supplementation affects calcium and phosphorus levels in the serum in rats.

II. Materials And Methods

The study was conducted in the Experimental Research Lab in the Department of Physiology, Jawaharlal Nehru Medical College, Bhagalpur. The ethical Clearance was approved for the study. The study included 30 adult rats of Sprague-Dawley species, which were allocated to three groups as follows:

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

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 the departmental lab.

Zinc sulphate administration

After being dissolved in distilled water, zinc sulphate was administered in 0.5 ml serum physiological in the form of 3 mg/kg/day intraperitoneal injections. Zinc sulphate injections were made at the same hour of the day (at 09:00 a.m.) for six weeks.

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 Jawaharlal Nehru Medical College, Bhagalpur using 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 by Absorption Spectrophotometer in the Biochemistry Department of Jawaharlal Nehru Medical College, Bhagalpur. Zinc levels were expressed as μg/dl.

III. Statistical Analyses

Statistical evaluation of the findings was made using SPSS 20 software Package. 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.

IV. 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 group fed on a zinc-deficient diet (group 3) was found significantly lower than those in groups 1, and 2 (p<0.05, table 1). Calcium and phosphorus levels in groups 1 and 2 were not different from each other, but were higher than those levels in group 3 (p<0.05). Group 3 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 (group 2) 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 2 (p<0.05), but higher than those in group 3 (p<0.05). Serum zinc levels in the zinc-deficient group were significantly lower than those in the remaining groups (p<0.05, table 2).

V. Discussion

Although mean weights of the groups at the beginning of the study were not different, it has been reported that zinc-deficient group 3 had a significant weight loss at the end of the study. It can be said that the weight loss observed in group 3 was an expected result, as it was demonstrated in several studies that zinc deficiency in the diet caused weight loss.

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 (16). 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 (6).
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>187.50±10.34</td>
<td>201.50±10.34</td>
<td>22.00±16.74</td>
</tr>
<tr>
<td>2 Zinc-supplemented</td>
<td>201.00±10.49</td>
<td>222.50±13.95</td>
<td>21.00±16.63</td>
</tr>
<tr>
<td>3 Zinc-deficient</td>
<td>201.50±15.14</td>
<td>187.50±12.03</td>
<td>14.00±10.55</td>
</tr>
<tr>
<td>P</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

Mean values within the same column have statistical significance (P<0.05)

Table 2: 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>11.3±4.30</td>
<td>5.74±0.55</td>
<td>3.6±0.11</td>
<td>92±30±65.52</td>
</tr>
<tr>
<td>2 Zinc-supplemented</td>
<td>11.95±4.11</td>
<td>5.73±0.65</td>
<td>3.6±0.21</td>
<td>130.20±11.50</td>
</tr>
<tr>
<td>3 Zinc-deficient</td>
<td>8.23±0.48</td>
<td>3.50±0.47</td>
<td>3.32±0.17</td>
<td>51.60±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 within the same column have statistical significance (P<0.05)

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 rats. In this study we obtained the highest serum calcium and phosphorus levels in the control group (group 1) and the zinc-supplemented group (group 2). The lowest serum calcium and phosphorus levels, on the other hand, were found in the group fed on a zinc-deficient diet (group 3).

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 (10). Additionally, (17) 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. 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 group (group 2). Similarly, the group that was fed on a zinc-deficient diet (group 3) had the lowest serum zinc levels.

It is recognized that ovarian hormone deficiency in meno pause stimulates bone loss. Ovariectomy can lead to osteoporosis by causing lack of estrogen (14). It was 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 estrogens (11) when compared to the controls and that there was a considerable relation between osteoporosis and zinc discharge (12). The same researchers (12) pointed out that determination of zinc levels could be crucial in the early diagnosis of osteoporosis.

VI. Conclusion

1. When compared to rats fed on a normal diet, rats fed on zinc deficient diet have a significant decline in calcium and phosphorus levels.
2. Zinc supplementation helps to maintain serum calcium and phosphorus levels at the normal level in rats.
3. Zinc supplementation to rats can be useful in restoring the calcium mechanism.

References


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