Zinc Deficiency Increases Serum Concentrations of Parathyroid Hormone through a Decrease in Serum Calcium and Induces Bone Fragility in Rats

Takako Suzuki1, Yasutaka Kajita2, Shin-ichi Katsumata1, Hiroshi Matsuzaki1 and Kazuharu Suzuki1

1Department of Nutritional Science, Faculty of Applied Bioscience, Tokyo University of Agriculture, 1–1–1 Sakuragaoka, Setagaya-ku, Tokyo 156–8502, Japan
2Department of Food Sciences, College of Life Science, Ibaraki Christian University, 6–11–1 Omika-machi, Hitachi, Ibaraki 319–1295, Japan

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Summary We hypothesized that a zinc-deficient diet alters the mineral (calcium, magnesium, and phosphorus) components of bones, as well as hormones related to bone remodeling, and negatively affects bone metabolism. Four-week-old male Wistar rats were randomly assigned to one of three groups for 4 wk: a zinc-adequate group (C, 30 ppm); a zinc-deficient group (ZD, 1 ppm); and a pair-fed group (PF, 30 ppm), which was pair-fed to the ZD group. Bone mineral density and bone mechanical properties were reduced in the ZD group compared to the C and PF groups. Compared with the C and PF groups, serum osteocalcin, a bone formation marker, was reduced in the ZD group. Conversely, urine deoxypyridinoline, a bone resorption marker, was increased in the ZD group compared to the C and PF groups. Calcium and phosphorus concentrations in bone were not different among all groups. The bone magnesium concentration was significantly higher in the ZD group than in the PF and C groups. Interestingly, compared with the C and PF groups, the ZD group showed a reduction in serum calcium concentration along with an increase in serum parathyroid hormone (PTH) concentration. Although serum 1,25-dihydroxycholecalciferol concentration was significantly higher in the ZD and PF groups than in the C group, the rate of apparent calcium absorption was significantly lower in the ZD group than in the C and PF groups. Therefore, zinc deficiency is suspected to cause an increase in serum PTH concentration owing to an inability to maintain calcium homeostasis, resulting in bone fragility.

Key Words zinc deficiency, calcium balance, bone metabolism, parathyroid hormone, 1,25-dihydroxycholecalciferol

Zinc is an essential nutrient for humans and animals that is involved in many physiological functions, including immune and antioxidant functions, growth, and reproduction. Considering the participation of zinc in a broad range of metabolic processes, zinc deficiency can lead to a variety of physiological and developmental impairments (1, 2). Zinc is relatively abundant in bone tissue, which contains approximately 30% of the total zinc in the body (3). It has been demonstrated that zinc stimulates osteoblastic bone formation and mineralization, inhibits osteoclastic bone resorption by inhibiting osteoclast-like cell formation from bone marrow cells, and stimulates the apoptotic cell death of mature osteoclasts in vitro (4–7). Therefore, zinc deficiency impairs bone growth, development, and the maintenance of bone health (8). Bone mass is maintained through bone remodeling that involves the formation and resorption of bone, which occur in two well-defined cellular events (9). Bone formation and resorption are complex biological processes and involve several regulated gene expression patterns of bone-related proteins. In addition, the dynamic and complex process of bone remodeling is tightly controlled by hormonal regulation (10, 11).

Calcium release requires bone destruction, and the principal mediators in this process are parathyroid hormone (PTH) and its downstream effector, 1,25-dihydroxycholecalciferol (1,25(OH)2D3). Therefore, calcium homeostasis is strictly regulated by PTH and 1,25(OH)2D3. PTH is expressed in the kidney and bone, and its synthesis and secretion are controlled by the serum calcium concentration and 1,25(OH)2D3. In bone, PTH promotes osteoclast formation and activity through the induction of receptor activator of nuclear factor-κB ligand (RANKL), which causes bone resorption and increases the serum calcium level (12). Furthermore, PTH from the parathyroid glands stimulates 1α-hydroxylation in the kidney. 1,25(OH)2D3 increases calcium absorption in the intestine by inducing an increase in expression of calcium transporter in the mucosa of the small intestine and duodenum. In addition, 1,25(OH)2D3 appears to facilitate bone formation at physiologically optimal concentrations. On the other hand, the higher levels of hormones support RANKL-mediated osteoclastogenesis in the same manner as
Zinc Deficiency and Bone Metabolism

1,25(OH)2 D3, and negatively affects bone metabolism. The hormones related to bone remodeling such as PTH and calcium, magnesium, and phosphorous in bone and a zinc-deficient diet alters the mineral components (e.g., contents, and the hormones related to bone remodeling, metabolism, including mechanical parameters, mineral content in bone are inconsistent. Nielsen found that a zinc-deficient diet increased calcium, magnesium, and phosphorus concentrations in the tibia of growing rats (20). In contrast, it has been reported that calcium and magnesium concentrations in the femurs of growing rats are not affected by dietary zinc deficiency (24, 25).

The relationship between zinc deficiency and bone metabolism, including mechanical parameters, mineral contents, and the hormones related to bone remodeling, is unclear. Based on the literature, we hypothesized that a zinc-deficient diet alters the mineral components (e.g., calcium, magnesium, and phosphorus) in bone and hormones related to bone remodeling such as PTH and 1,25(OH)2D3, and negatively affects bone metabolism. The purpose of this study was to clarify the interactions of bone minerals with bone metabolism in zinc-deficient rats.

**MATERIALS AND METHODS**

**Animals and diets.** Four-week-old male Wistar rats were purchased from CLEA Japan, Inc. (Tokyo, Japan) and cared for according to the Guide for the Care and Use of Laboratory Animals of the Tokyo University of Agriculture Animal Use Committee. The rats were individually housed in stainless steel wire-bottom cages in a temperature-controlled room at 22 ± 0.5°C with an alternating 12-h light and dark cycle. After a 3-d adaptation period with a nutritionally complete control basal diet by the addition of a zinc-free AIN-93G mineral mixture instead of the AIN-93G mineral mixture. The rats in two of the three groups had free access to a control diet (C group) or a zinc-deficient diet (ZD group). The rats in the third group (PF group) were pair-fed with the control diet to the mean intake of the ZD group. The experimental diets were based on the AIN-93G diet (26) with egg albumin as the protein source (Table 1). The zinc-deficient diet was prepared from a basal diet by the addition of a zinc-free AIN-93G mineral mixture instead of the AIN-93G mineral mixture. The zinc concentrations of the control and zinc-deficient diets were 30 and 1 ppm, respectively. The rats were fed their respective diets and provided free access to distilled water for 4 wk. Feed intake was measured daily, and body weight was recorded. Feed efficiency was calculated as the weight gain/feed intake. Rat feces and urine were collected for the last 3 d to assess zinc, calcium, and magnesium absorption. Urine was collected over 24 h prior to euthanasia for the measurement of bone resorption markers.

At the end of the experiment, the rats were food-deprived for 12 h and then anesthetized using pentobarbital. Blood was collected from the abdominal aorta using a heparinized syringe and centrifuged at 3,000 rpm for 15 min to obtain plasma. Serum and urine were stored at −80°C until analysis.

**Biochemical analyses.** Feces were dried, weighed, and milled. Powdered fecal samples and urine were demineralized with a 1-mol/L HCl solution. Serum zinc was measured using a metalloassay kit (Metallogenics Co., Ltd., Chiba, Japan). Other minerals in sera, fecal samples, urine, and bone were analyzed by atomic absorption spectrophotometry (Hitachi A-2000, Tokyo, Japan) according to the method of Gimblet et al. (27). Bone phosphorus was analyzed colorimetrically according to the method of Gomori (28). Apparent absorption and retention of calcium, magnesium, and iron were calculated as intakes, fecal excretion, and urinary excretion. Serum PTH concentrations were measured using a rat intact PTH ELISA kit (Immutopics, San Clemente, CA). Serum 1,25(OH)2D3 concentrations were measured using a 1,25(OH)2 vitamin D EIA kit (Immundiagnos-

**Table 1. Compositions of the experimental diets.**

<table>
<thead>
<tr>
<th>Chemical analysis</th>
<th>Control diet</th>
<th>Zinc deficient diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn level (%)</td>
<td>0.003</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ca level (%)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Mg level (%)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>P level (%)</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Amount of energy (kJ/kg)</td>
<td>943</td>
<td>943</td>
</tr>
<tr>
<td>Egg albumin</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>529.486</td>
<td>529.486</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Mineral mixture1</td>
<td>35.0</td>
<td>35.01</td>
</tr>
<tr>
<td>Vitamin mixture2</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>l-Cystine</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>tert-butylhydroquinone</td>
<td>0.014</td>
<td>0.014</td>
</tr>
</tbody>
</table>

1. AIN-93G mineral mixture.
2. AIN-93 vitamin mixture.
3. The mineral mixture is a modification of AIN-93G mineral mixture without zinc carbonate.
The serum osteocalcin concentration was measured using the Osteocalcin Rat ELISA system (GE Healthcare Bio-Sciences, Little Chalfont, UK). Urinary deoxypyridinoline (DPD) excretion was measured using a METRA DPD EIA kit (Quidel, San Diego, CA). Urinary creatinine excretion was measured using a creatinine test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Bone mechanical parameters. Femurs were removed from each rat and stored at 5˚C until analysis. Bone mineral content (BMC) and bone mineral density (BMD) were measured by dual-energy X-ray absorptiometry (DXA; DCS-600EX-R; Aloka, Tokyo, Japan). After BMC and BMD analyses, the femurs were analyzed to determine their breaking strength using a 5-kN flexure fixture configured for three-point bend tests. The crosshead speed was 10 mm/min. Breaking energy and force were determined in femurs using a bone strength tester, model TK252C (Muromachi Kikai, Tokyo, Japan).

Statistics. Data are expressed as means±standard error of the mean (SE). The significance of differences among the groups was determined by one-way analysis of variance and Fisher’s protected least-significant difference. Differences were considered significant at p<0.05.

RESULTS

Food intake and growth rate

Food intake was significantly lower in the ZD and PF groups than in the C group, and there were no differences between the ZD and PF groups (Table 2). The final body weight, body weight gain, and food efficiency were significantly lower in the ZD group than in the C and PF groups, and they were significantly lower in the PF group than in the C group.

Amount and rate of apparent absorption and retention of zinc, calcium, and magnesium

The amount of apparent zinc absorption was significantly lower in the ZD group than in the C and PF groups, and significantly lower in the PF group than in the C group (Table 3). The rate of apparent zinc absorption was not different among any of the groups. More-
over, the amount of zinc retention was significantly lower in the ZD group than in the C and PF groups, and significantly lower in the PF group than in the C group. The rate of zinc retention was significantly lower in the ZD group than in the C and PF groups, with no difference between the C and PF groups. The amount of apparent calcium absorption was significantly lower in the ZD and PF groups than in the C group, with no difference between the ZD and PF groups. In addition, the rate of apparent calcium absorption was significantly lower in the ZD group than in the C and PF groups, with no difference between the C and PF groups. The amount of calcium retention was significantly lower in the ZD and PF groups than in the C group, with no difference between the ZD and PF groups. The rate of calcium retention was not different among any of the groups. The amount of apparent magnesium absorption was significantly lower in the ZD and PF groups than in the C group, with no difference between the ZD and PF groups. On the other hand, the rate of apparent magnesium absorption was significantly higher in the ZD and PF groups than in the C group, with no difference between the ZD and PF groups. Moreover, the amount of magnesium retention was significantly lower in the ZD and PF groups than in the C group, with no difference between the ZD and PF groups. The rate of magnesium retention was not different among any of the groups.

**Mineral concentrations in serum**

The serum zinc concentration was significantly lower in the ZD group than in the C and PF groups, with no difference between the C and PF groups (Table 4). There was no significant difference in serum magnesium concentrations between the C and ZD groups. However, the serum magnesium concentration was higher in the ZD group than in the PF group. Conversely, the serum calcium concentration was significantly lower in the ZD group than in the C and PF groups, with no difference between the C and PF groups.

**Bone parameters**

Bone length was significantly lower in the ZD group than in the C and PF groups, with no difference between the ZD and PF groups (Table 5). In addition, the bone area (BA) and BMC of the femur were significantly lower in the ZD group than in the C and PF groups, with no difference between the C and PF groups. The breaking

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### Table 4. Serum zinc, magnesium, and calcium concentrations in the C, PF, and ZD groups.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>PF</th>
<th>ZD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc (μmol/L)</td>
<td>24.38±1.05a</td>
<td>22.63±0.63a</td>
<td>10.23±0.65b</td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>0.93±0.03ab</td>
<td>0.86±0.03a</td>
<td>1.00±0.04b</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.40±0.03a</td>
<td>2.43±0.02a</td>
<td>2.24±0.05b</td>
</tr>
</tbody>
</table>

Values are the mean±SE for 8–10 rats. Mean values in a row without a common superscripted letter differ significantly, p<0.05.

### Table 5. Length, weight, BA, BMC, BMD, mechanical strength, mineral concentrations, and contents of the femur in the C, PF, and ZD groups.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>PF</th>
<th>ZD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone length (cm)</td>
<td>3.06±0.02a</td>
<td>2.84±0.03b</td>
<td>2.85±0.03b</td>
</tr>
<tr>
<td>Bone weight (g)</td>
<td>0.34±0.01a</td>
<td>0.29±0.003b</td>
<td>0.28±0.01c</td>
</tr>
<tr>
<td>BA (cm²)</td>
<td>1.66±0.01a</td>
<td>1.56±0.02b</td>
<td>1.57±0.02b</td>
</tr>
<tr>
<td>BMC (mg)</td>
<td>166.21±1.61a</td>
<td>151.35±1.86b</td>
<td>145.21±3.62b</td>
</tr>
<tr>
<td>BMD (mg/cm³)</td>
<td>99.11±0.23a</td>
<td>97.30±1.16a</td>
<td>92.66±1.64b</td>
</tr>
<tr>
<td>Breaking energy (mJ)</td>
<td>10.64±0.72a</td>
<td>7.94±0.74b</td>
<td>6.39±0.60b</td>
</tr>
<tr>
<td>Breaking force (N)</td>
<td>55.85±3.43a</td>
<td>51.39±3.12a</td>
<td>42.52±1.92b</td>
</tr>
<tr>
<td>Zinc (mg/g dry weight)</td>
<td>0.39±0.01a</td>
<td>0.39±0.01a</td>
<td>0.08±0.002a</td>
</tr>
<tr>
<td>Calcium (mg/g dry weight)</td>
<td>189.17±2.99a</td>
<td>196.49±2.84a</td>
<td>194.59±1.55a</td>
</tr>
<tr>
<td>Magnesium (mg/g dry weight)</td>
<td>3.45±0.10a</td>
<td>3.72±0.05b</td>
<td>4.20±0.02c</td>
</tr>
<tr>
<td>Phosphorus (mg/g dry weight)</td>
<td>99.56±1.12a</td>
<td>100.10±0.75a</td>
<td>99.89±0.58a</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>0.13±0.002a</td>
<td>0.11±0.001b</td>
<td>0.02±0.00c</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>63.60±1.74a</td>
<td>56.35±1.13b</td>
<td>52.36±0.83c</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>1.16±0.04a</td>
<td>1.07±0.01b</td>
<td>1.15±0.02a</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>33.46±0.75a</td>
<td>28.70±0.35b</td>
<td>27.09±0.40c</td>
</tr>
</tbody>
</table>

1 BA: bone area, 2 BMC: bone mineral content, 3 BMD: bone mineral density.

Values are the mean ±SE for 8–10 rats. Mean values in a row without a common superscripted letter differ significantly, p<0.05.
energy of the femur was significantly lower in the ZD and PF groups than in the C group, with no difference between the ZD and PF groups. The breaking force of the femur was significantly lower in the ZD group than in the C and PF groups, with no difference between the C and PF groups.

**Bone mineral concentrations and contents**

The zinc concentration in the femur was significantly lower in the ZD group than in the C and PF groups, with no difference between the C and PF groups (Table 5). Calcium and phosphorus concentrations in the femur were not different among any of the groups. On the other hand, the magnesium concentration in the femur was significantly higher in the ZD group than in the PF and C groups, and significantly higher in the PF group than in the C group. The contents of zinc, calcium, and phosphorus in the whole femur were significantly lower in the ZD group than in the PF and C groups, and significantly lower in the PF group than in the C group. On the other hand, there was no significant difference in the magnesium content of the whole femur between the C and ZD groups. However, the magnesium content of the whole femur was higher in the ZD group than in the PF group.

**Markers of bone turnover**

The serum osteocalcin concentration was significantly lower in the ZD group than in the C and PF groups, with no difference between the C and PF groups (Fig. 1). Urinary DPD excretion was significantly higher in the ZD group than in the C and PF groups, and significantly higher in the PF group than in the C group.

**Serum PTH and 1,25(OH)2D3 concentrations**

The serum PTH concentration was significantly higher in the ZD group than in the C and PF groups, with no difference between the C and PF groups (Fig. 2). The serum 1,25(OH)2D3 concentration was significantly higher in the ZD and PF groups than in the C group, with no difference between the ZD and PF groups.

**DISCUSSION**

Numerous studies have shown evidence of the important relationship between adequate zinc intake and bone health, and that low dietary levels and plasma concentrations of zinc are associated with increased osteopenia, osteoporosis, and fracture risk in humans and animals (4–8, 29–33). However, no studies have documented the relationship between zinc deficiency and bone metabolism, including mechanical parameters, mineral contents, and the hormones related to bone remodeling. In this study, it was found that a zinc-deficient diet alters the mineral components of bone and negatively affects bone metabolism in rats. In addition, zinc-deficient rats showed deficiencies in proteins and other minerals that are important in bone metabolism.

We assumed that apparent zinc absorption would increase, because the requirement for zinc rises in rats...
fed a zinc-deficient diet. However, the rate of apparent zinc absorption was not different among any of the groups in this study even though zinc intake was significantly lower in the ZD group than in the C and PF groups. Furthermore, the rate of zinc retention was significantly lower in the ZD group than in the C and PF groups. These findings suggest that the zinc-deficient diet had a severe effect on the rats, because other studies have reported that severe zinc deficiency induces structural and functional alterations in the intestines of rats by oxidative damage (34).

In this study, femoral calcium and phosphorous concentrations were not different among any of the groups, but zinc deficiency decreased BMD, which is evaluated by BMC and bone area. In the present study, zinc deficiency decreased the calcium and phosphorous contents of the whole femur. Furthermore, it appeared that zinc deficiency decreased BMD by decreasing the calcium and phosphorous contents of the whole femur. In addition, the present study demonstrated that zinc deficiency decreases the mechanical strength of bone. Bone strength reflects not only BMD, but also bone quality, which is related to bone architecture, turnover, damage accumulation, and mineralization. Therefore, the present results suggest that dietary zinc deficiency might not only reduce BMC, but also damage bone tissues. Bone mass is maintained by repeated cycles of destruction and rebuilding to maintain the balance between bone formation and resorption, which are controlled by osteoblasts and osteoclasts, respectively (9).

Osteocalcin is a major noncollagenous protein of the bone matrix, which is synthesized and released from osteoblasts (35). Therefore, the serum osteocalcin concentration is used as a biochemical marker of bone formation. In this study, the serum osteocalcin concentration was lower in the ZD group than in the C and PF groups. Zinc is an important factor in bone formation because zinc-dependent enzymes, including alkaline phosphatase, which are found on the surface of osteoblasts, are essential for normal bone formation and/or mineralization (36). Furthermore, numerous studies have demonstrated that zinc deficiency is associated with metabolic disturbances of enzymes involved in bone development (25, 37–39). In addition, it has been reported that Runx2, which has a major impact on osteogenesis by stimulating the gene expression of osteocalcin, alkaline phosphatase, and collagen type 1, was decreased with zinc deficiency in vitro (40). These reductions are known to decrease bone matrix and mineralization. Therefore, we suggest that zinc is directly involved in bone formation, and that zinc deficiency results in decreases in bone formation and osteoblastic differentiation and activity.

To assess bone resorption, urinary excretion of DPD was examined as a biochemical marker of bone resorption. DPD is degraded from type I collagen during osteoclastic bone resorption, and urinary excretion of DPD is useful to determine changes in bone resorption (41, 42). In the present study, dietary zinc deficiency increased urinary DPD excretion, as shown in comparisons of the ZD group with the C and PF groups. Several studies have reported that a zinc-deficient diet does not affect bone resorption (25, 38, 43). The difference in bone resorption between the present study and other studies might be explained by the degree of zinc deficiency, the experimental design, and the different compositions of experimental diets. In the bone marrow, zinc deficiency increases the induction of 8-hydroxy-2′-deoxyguanosine, a marker of cellular DNA damage by oxidative stress, through an increase in the biological action of superoxide radicals (44). Furthermore, it is known that the generation of oxygen-derived free radicals in the bone environment causes osteoclast formation and bone resorption in vitro and in vivo (45). These studies support the notion that zinc deficiency induces an increase in bone resorption, as shown in our study. Therefore, a zinc-deficient diet decreases bone formation and increases bone resorption, which cause osteoporosis.

In this study, it was clearly shown that a low zinc diet changes the mineral distribution that is closely associated with bone metabolism. The serum calcium concentration was remarkably lower in the ZD group than in the C and PF groups, leading to subsequent stimulation of PTH secretion. Circulating PTH levels are affected by the serum concentration of calcium, which is the most important regulator of PTH secretion (12). Secreted PTH has been proposed as a tropic factor in the modulation of 1,25(OH)2 D3 synthesis and is thus a factor responsible for the adaptation of intestinal calcium transport to variations in the serum calcium concentration. In terms of the action of PTH on bone, elevated PTH secretion causes an increase in bone resorption. DPD is degraded from type I collagen when bone tissue is broken down during bone remodeling. Therefore, the urinary excretion of DPD is a suitable index for PTH activity in bone. In addition, it has been shown that the generation of oxygen-derived free radicals is associated with osteoclastic bone resorption stimulated by PTH (45). Hence, these studies suggest that dietary zinc deficiency might induce bone resorption through an increase in the action of PTH, resulting in a decrease in BMC and BMD.

In this study, the serum 1,25(OH)2 D3 concentration was significantly higher in the ZD and PF groups than in the C group. 1,25(OH)2 D3 synthesis is stimulated by PTH from the parathyroid glands and inhibited by the level of free calcium ions in the blood. Moreover, it is probable that 1,25(OH)2 D3 synthesis leads to vitamin D-dependent enhancement of intestinal calcium absorption (13, 14). However, the rate of apparent calcium absorption was significantly lower in the ZD group than in the C and PF groups without increases in serum 1,25(OH)2 D3 concentrations. These results suggest that a zinc-deficient diet causes an impairment in calcium utilization that is not regulated by increases in serum PTH or 1,25(OH)2 D3 concentrations. In addition, this impairment in calcium utilization is suggested to lead to a decrease in serum calcium concentration, resulting in an increase in PTH secretion. Several studies have
reported that intestinal absorption of zinc and calcium is antagonistic: a low calcium diet mobilizes zinc from the bones and increases zinc absorption, and a high calcium diet decreases zinc absorption (21). Therefore, zinc deficiency is predicted to increase intestinal calcium absorption. However, in this study, the ZD group showed a significantly reduced rate of apparent calcium absorption despite increases in PTH and 1,25(OH)₂D₃ concentrations.

On the other hand, the PF group showed an increase in the serum 1,25(OH)₂D₃ concentration despite a lack of alteration in the serum PTH concentration. In addition, serum calcium concentration and calcium absorption were normally regulated in the PF group, but not in the ZD group. Therefore, we assumed that in the PF group, calcium resorption was increased by increasing serum 1,25(OH)₂D₃ concentrations to maintain bone metabolism. However, the exact mechanism underlying this regulation remains to be determined. In the present study, food intake was suspected to decrease dramatically in the ZD group, because it has been reported that a zinc-deficient diet causes severe growth retardation and a reduction in food intake (46, 47). As the PF group was fed according to the food intake of the ZD group, the intake of the PF group was thought to be severely limited; as a result, the PF group appeared to change its requirements for several minerals and a stress response was triggered. Therefore, both a pair-feeding group and an ad-libitum group were provided in this study. Compared to the C group, the PF group had more magnesium absorption, a greater magnesium concentration in bone, and increased serum 1,25(OH)₂D₃ concentrations to maintain calcium metabolism. Accordingly, the severely restricted feeding such as in the PF group seems to have had some effect on mineral metabolism. However, further studies are needed to determine why the severely restricted feeding changed the metabolism of several minerals and bone.

In bone, 1,25(OH)₂D₃ appears to facilitate bone formation at physiologically optimal concentrations, while higher levels of 1,25(OH)₂D₃ promote resorption and limit mineralization to sculpt bone. In the present study, the serum 1,25(OH)₂D₃ level and urinary DPD excretion were both suitable indices for PTH activity in the ZD group. Accordingly, we considered that the decrease in serum calcium concentration might be a factor responsible for the BMD decrease in the ZD group. In contrast to the ZD group, the PF group maintained BMD despite an increase in the serum 1,25(OH)₂D₃ level in this study. These alterations in serum 1,25(OH)₂D₃ concentrations were thought to be caused by different factors, even though the serum 1,25(OH)₂D₃ level was elevated in not only zinc-deficient rats, but also in pair-fed rats. However, further studies are needed to clarify the different mechanisms for the elevated serum 1,25(OH)₂D₃ level in zinc-deficient rats and pair-fed rats.

This study showed that a zinc-deficient diet alters the mineral components in bone and negatively affects bone metabolism in rats. Zinc deficiency increased magnesium concentrations in bone. Interestingly, it has been shown that dietary magnesium deficiency significantly increases intestinal zinc absorption and zinc concentrations in the femur (22). On the other hand, little has been reported on the bioavailability of magnesium and magnesium concentrations of bone with a zinc-deficient diet, and inconsistent results have been seen. Salgueiro et al. (24) reported that severe and marginal zinc deficiency did not affect magnesium concentrations of the femur. In contrast, Nielsen (20) noted that marginal zinc deficiency significantly decreased magnesium excretion and increased magnesium concentrations in bone. These findings suggest a possible interaction between zinc and magnesium. Furthermore, it is known that magnesium deficiency leads to a reduction in bone mass, abnormal bone growth, and an increase in skeletal fragility in animal models (48–50). Epidemiologic studies have demonstrated that dietary magnesium intake correlates with bone density (51, 52). In postmenopausal osteoporotic women, it has been demonstrated that daily oral magnesium supplementation promotes bone formation and suppresses bone resorption (53). Therefore, these studies might imply that the increase in the magnesium concentration in bone caused by zinc deficiency is a positive function related to bone formation or absorption. However, further studies are needed to determine why the magnesium concentration in bone was increased in the ZD group and whether an increase in the magnesium concentration in bone contributes to osteoporosis induced by zinc deficiency.

In this study, we hypothesized that a zinc-deficient diet alters the mineral components, such as calcium, magnesium, and phosphorous, in bone and negatively affects bone metabolism. This study demonstrated that zinc deficiency directly gives rise to a reduction in bone formation and is indirectly involved in an increase in bone absorption through an alteration of calcium metabolism, resulting in a significant loss of bone. In addition, zinc deficiency gives rise to a decrease in the serum calcium level caused by an impairment of calcium utilization, and leads to an increase in secretion of PTH. Therefore, we suggest that a decrease in the rate of calcium absorption might be a trigger for the promotion of bone absorption caused by zinc deficiency. In conclusion, dietary zinc deficiency causes decreased bone formation and secondary hyperparathyroidism that increases bone resorption, resulting in bone fragility. Furthermore, the intestinal absorption of calcium is not normally regulated by PTH or 1,25(OH)₂D₃ secretions in zinc-deficient rats.

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REFERENCES

2) Prasad AS. 1991. Discovery of human zinc deficiency


