STIMULATORY EFFECT OF ZINC ON BONE GROWTH IN WEANLING RATS

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The effect of zinc on bone metabolism was investigated in weanling rats orally administered zinc sulfate (0.1, 1.0 and 10 mg Zn/100 g body wt) for 30 d. The administration of zinc produced dose-dependent increases in zinc contents of the femoral diaphysis and epiphysis. The dry weight of the femur was significantly increased by the doses of 0.1 and 1.0 mg Zn/100 g for 3 d, while significant reduction of that was observed by zinc administration for 30 d. Meanwhile, DNA content, calcium content and alkaline phosphatase activity in the femoral diaphysis and epiphysis were significantly increased by the doses of 0.1 and 1.0 mg Zn/100 g for 7 d. However, by the 30 d administration of zinc (0.1, 1.0 and 10 mg/100 g), calcium contents in the femur and serum were markedly decreased. These results suggest that comparatively low dose of zinc may stimulate the bone growth and calcification of weanling rats.

Keywords—zinc; bone; calcium; DNA; alkaline phosphatase

INTRODUCTION

Zinc has been demonstrated to have a wide variety of roles in mammalian system.1) Zinc may play a physiological role in the growth and calcification of bone tissue.2) Zinc-deficient rats have decreases in calcium balance, and rate of bone anabolism and resorption.3) Zinc is also required for the complete physiological action of vitamin D, a stimulating factor on bone calcification, on calcium metabolism in rats.4)

On the contrary, zinc deficiency does not affect calcium and phosphorus depositions into bone.5) High zinc diet exert a porotic effect in bone.6) Also, high dose of zinc tends to inhibit bone collagen synthesis in young animals7) and causes bone resorption related to the abnormality of calcium metabolism in rats.8,9)

Thus the role of zinc in bone metabolism has not been fully solved. The present study was therefore, designed to clarify a dose-related physiological role of zinc in the bone growth of weanling rats. It was found that comparatively low dose of zinc stimulated the bone growth and calcification of weanling rats.

MATERIALS AND METHODS

Weanling male Wistar rats (3 weeks old) weighing 67—71 g were obtained from the Nippon Bio Supp. Center Co., Tokyo. The animals were fed commercial laboratory chow containing 1.1% Ca, 1.1% P and 0.012% Zn, and distilled water freely. Zinc sulfate was dissolved in distilled water to concentrations of 0.1, 1.0 and 10 mg as Zn per ml. These solutions (10 ml/100 g body wt) were orally administered to rats for 3, 7, 15 and 30 d. The animals were bled by cardiac puncture 24 h after the last administration of zinc, and the blood and femur removed. The serum was separated and analyzed immediately. The femur was soaked in cold 0.25M sucrose solution.

Serum calcium was determined by atomic absorption spectrophotometry after precipitation of protein.10) Serum inorganic phosphorus and alkaline phosphatase activity were determined by the methods of Taussky and Shon,11) and Kind and King,12) respectively.
The femur was cleaned of soft tissue and marrow, and the diaphysis and the epiphysis (containing metaphyseal bone tissue) were separated, ashed for 24 h at 640°C, weighed, and dissolved in 6 N HCl. Calcium was determined by atomic absorption spectrophotometry. Inorganic phosphorus was measured by the method of Nakamura and Mori.\textsuperscript{13} The femur was extracted with mixture of 100% ethanol and diethyl ether (1:1) for 24 h at 0°C.\textsuperscript{14} After drying at 95-100°C for 24 h the bone tissue was weighed to obtain a dry weight. Zinc content in the bone was determined by atomic absorption spectrophotometry after digestion with nitric acid and was expressed as the amount of zinc (\(\mu g\)) per g dry weight of bone tissue.

The femoral diaphysis and epiphysis fragment were shaken separately with 4 ml of 0.1 N NaOH solution for 16 h at 0°C.\textsuperscript{15} After alkali extraction, the samples were centrifuged at 10,000 \(\times\) g for 10 min, and the supernatant collected. DNA content was determined by the method of Ceriotti\textsuperscript{16} and was expressed as the amount of DNA (mg) per g wet weight of bone tissue.

The femoral diaphysis and epiphysis tissues were immersed in 3 ml of 6.5 mM barbital buffer (pH 7.4) at 0°C, and were disrupted for 20 s with an ultrasonic device.\textsuperscript{17} The supernatant centrifuged at 6000 \(\times\) g for 5 min was used for measurement of the enzyme activities. Acid and alkaline phosphatase activities were determined by the method of Lindhardt and Walter.\textsuperscript{18} Enzyme activity was expressed as nmol of \(p\)-nitrophenol liberated per min per mg protein. Protein was determined by the method of Lowry et al.\textsuperscript{19}

The data were subjected to analysis of variance, and the S.E. was calculated from the residual error term. Statistical significance is expressed as \(p\) values from Student's \(t\)-test.

RESULTS

Effect of Zinc Administration on Bone Weight

![Graph showing change of zinc content in diaphysis and epiphysis over time](image)

**FIG. 1. Change of Zinc Content in the Femur of Rats orally Administered Zinc Sulfate for 3, 7, 15 and 30 d**

The rats were killed 24 h after the last administration of zinc sulfate (0.1, 1.0 and 10 mg Zn/100 g). Each point is the mean of six animals. Vertical lines represent the S.E.M. Key: a) \(p < 0.01\), compared with the control; (--- O ---) control; (--- ▲ ---) 0.1 mg Zn/100 g; (--- ● ---) 1.0 mg Zn/100 g; (--- ■ ---) 10 mg Zn/100 g.
The administration of zinc produced progressive elevation of zinc content in the femoral diaphysis and epiphysis with a dose-dependence of the metal (Fig. 1). The accumulation of zinc into the femoral diaphysis and epiphysis was markedly increased at the dose of 10 mg Zn/100 g.

The change of the dry weight of femur by the administration of zinc to rats for 30 d is shown in Fig. 2. The dry weight of the femoral tissue was significantly increased by the administration of zinc (0.1 and 1.0 mg/100 g) for 3 d, and a slight increase was observed at the dose of 10 mg/100 g. However, all the doses of zinc for 30 d caused significant decreases of the bone weight.

Effect of Zinc Administration on Bone DNA Content

DNA contents in the femoral diaphysis and epiphysis were markedly increased by the dose of 1.0 mg Zn/100 g for 3 and 15 d, while those were raised slightly but not significantly by the dose of 10 mg Zn/100 g for 3 d (Fig. 3). However, the dose of 10 mg Zn/100 g for 15 d caused remarkable decreases of DNA contents in the femoral diaphysis and epiphysis.

Effect of Zinc Administration on Bone Mineral Compositions

The calcium contents in the femoral diaphysis and epiphysis were significantly increased by the doses of 0.1 and 1.0 mg Zn/100 g for 3 d, although only a slight increase was observed in the case of 10 mg Zn/100 g (Fig. 4). However, all the doses of zinc for 15 and 30 d produced remarkable decreases of calcium content in the femoral diaphysis and epiphysis.

Meanwhile, phosphorus contents in the femoral diaphysis and epiphysis were not significantly altered by the administration of zinc for 3 to 30 d (Fig. 5).

Effect of Zinc Administration on Bone Phosphatase Activities

Alkaline phosphatase activities in the femoral diaphysis and epiphysis were markedly raised by the administration of zinc for 3 d at the dose of 1.0 mg/100 g but not at 10 mg/100 g (Fig. 6). The enzyme activities were not significantly altered by the administration of zinc for 15 d at both the doses.

Acid phosphatase activities in the femoral diaphysis and epiphysis were significantly decreased by the administration of zinc at the dose of 10 mg/100 g for 3 d, although those decreases were not seen in the case of 1.0 mg Zn/100 g (Fig. 7). Meanwhile, the administration of zinc (10 mg/100 g) for 15 d caused a significant increase in the enzyme activity in the femoral epiphysis.

Changes of Serum Mineral Compositions by Zinc Administration

The effects of increasing doses of zinc (0.1, 1.0 and 10 mg/100 g) on calcium and inorganic phosphorus concentrations, and alkaline phoshpatase activity in the serum of rats orally administered zinc sulfate are shown in Fig. 8. The serum calcium concentration was not significant-

![Graph](image-url)

**FIG. 2. Effect of Zinc on the Dry Weight of the Femur in Rats**

The rats were killed 24 h after the oral administration of zinc sulfate (0.1, 1.0 and 10 mg Zn/100 g) for 3, 7, 15 and 30 d. Each point is the mean of six animals. Vertical lines represent the S.E.M. Key: a) p < 0.01, compared with the control; (--- ○ ---) control; (--- ▲ ---) 0.1 mg Zn/100 g; (--- ● ---) 1.0 mg Zn/100 g; (--- ■ ---) 10 mg Zn/100 g.
FIG. 3. Effect of Zinc on DNA Content of the Femur in Rats
The rats were killed 24 h after the oral administration of zinc sulfate (1.0 and 10 mg Zn/100 g) for 3 and 15 d. Each bar is the mean of six animals. Vertical lines represent the S.E.M. Key: a) p < 0.01, compared with the control; (□) control; (■) 1.0 mg Zn/100 g; (□) 10 mg Zn/100 g.

FIG. 4. Effect of Zinc on Calcium Content of the Femur in Rats
The rats were killed 24 h after the oral administration of zinc sulfate (0.1, 1.0 and 10 mg Zn/100 g) for 3, 7, 15 and 30 d. Each point is the mean of six animals. Vertical lines represent the S.E.M. Key: a) p < 0.01, compared with the control; (○) control; (▲) 0.1 mg Zn/100 g; (●) 1.0 mg Zn/100 g; (■) 10 mg Zn/100 g.
ly altered by the administration of zinc at the dose of 0.1 and 1.0 mg/100 g for 3 d, while the dose of 10 mg Zn/100 g produced a significant decrease of serum calcium level. By the administration of zinc for 30 d, significant reductions of the serum calcium concentration were observed at all doses.

**FIG. 5. Effect of Zinc on Phosphorus Content of the Femur in Rats**

The rats were killed 24 h after the oral administration of zinc sulfate (0.1, 1.0 and 10 mg Zn/100 g) for 3, 7, 15 and 30 d. Each point is the mean of six animals. Vertical lines represent the S.E.M. (--- ○ ---) control; (— ▲ —) 0.1 mg Zn/100 g; (— ● —) 1.0 mg Zn/100 g; (— ■ —) 10 mg Zn/100 g.

**FIG. 6. Effect of Zinc on Alkaline Phosphatase Activity of the Femur in Rats**

The rats were killed 24 h after the oral administration of zinc sulfate (1.0 and 10 mg Zn/100 g) for 3 and 15 d. Each bar is the mean of six animals. Vertical lines represent the S.E.M. Key: a) $p < 0.01$, compared with the control; (□) control; (■) 1.0 mg Zn/100 g; (◇) 10 mg Zn/100 g.
of zinc. Significant decreases of the serum inorganic phosphorus concentration were also found only at the dose of 10 mg Zn/100 g for 7 and 15 d.

Meanwhile, alkaline phosphatase activity in the serum was significantly increased by all doses of zinc for 3 d. However, no increases were seen at 7, 15 and 30 d, although the dose of 10 mg Zn/100 g for 7 d caused a significant increase in the enzyme activity.

DISCUSSION

It has been suggested that, by using zinc-deficient animals, zinc may play a physiological role in growth and calcification in bone tissue.\(^{2-4}\) The mechanism of action of zinc on bone metabolism, however, has not been clarified.

The present findings, that the oral administration of zinc (1.0 mg/100 g body weight) to weanling rats for 3 d produces significant increases in the dry weight of bone tissue, DNA content, calcium content and alkaline phosphatase activity in the femoral diaphysis and epiphysis, suggest that short term administration of comparatively low dose of zinc may stimulate the growth and calcification of bone in weanling rats. However, prolonged administration of zinc may inhibit the growth and calcification of rat bone, since remarkable decreases in the dry weight of bone tissue and calcium content in the femur were observed by the administration of zinc (0.1, 1.0 and 10 mg/100 g) for 30 d.

DNA contents in the femoral diaphysis and epiphysis were markedly increased by short term administration of low dose of zinc, suggesting that zinc accumulated in the bone cells may stimulate the synthesis of DNA in the cells. Presumably zinc increased in the bone cells may activate DNA polymerase, since the enzyme might be a zinc-enzyme.\(^{20}\) The increase in DNA content of the bone tissue by zinc administration may lead to the proliferation of bone cells in weanling rats. Meanwhile, prolonged administration of high dose of zinc caused a remarkable decrease in

![Diagram](https://example.com/diagram.png)

**FIG. 7. Effect of Zinc on Acid Phosphatase Activity of the Femur in Rats**

The rats were killed 24 h after the oral administration of zinc sulfate (1.0 and 10 mg Zn/100 g) for 3 and 15 d. Each bar is the mean of six animals. Vertical lines represent the S.E.M. Key: a) \( p < 0.01 \), compared with the control; (□) control; (■) 1.0 mg Zn/100 g; (□) 10 mg Zn/100 g.
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DNA content. Perhaps, prolonged administration of zinc may reveal a toxic effect on bone cells, due to development of zinc accumulation into bone cells.

Bone mainly consists of inorganic matrix. The administration of zinc to weanling rats at early stage produced the increases in calcium contents in the femoral diaphysis and epiphysis but not phosphorus contents, suggesting that zinc may stimulate bone calcification in such a stage. Alkaline phosphatase in the bone cells participates in bone calcification.21 The enzyme activity was clearly enhanced by zinc administration. Presumably the increase in the femoral calcium content is partly based on the enhancement of the alkaline phosphatase activity, although the increase in collagen, main component of organic matrix in the bone tissue, initiates bone calcification.22 The mechanism of zinc action on the protein synthesis in the bone cells remains to be elucidated.

However, prolonged administration of zinc caused undesirable effect on bone calcium. The administration of zinc (0.1, 1.0 and 10 mg/100 g) to weanling rats for 15 and 30 d caused remarkable decreases of calcium content in the femoral diaphysis and epiphysis. The serum calcium concentration, in this case, was significantly lowered by zinc administration. From these results, it was assumed that the decrease of bone calcium by zinc administration might be related to a mechanism to maintain calcium homeostasis in the blood. The hypocalcemia stimulates secretion of parathyroid hormone from parathyroid glands. This hormone causes the mobilization of calcium into blood from bone by stimulating the release of lysosomal acid hydrolases from the bone cells.23 In the present experiment, the activity of acid phosphatase, a lysosomal enzyme, in the femoral epiphysis was significantly increased by the dose of 10 mg Zn/100 g for 15 d but not 1.0 mg Zn/100 g, although the administration of 10 mg Zn/100 g for 3 d caused significant decreases in the serum calcium concentration and the femoral acid phosphatase activity. At least partly, the progressive decrease in the femoral calcium by prolonged administration of zinc may result from a stimulation of bone resorption involved in parathyroid hormone. Meanwhile, slight increases but not significant of the alkaline phosphatase activities in the femoral diaphysis and epiphysis were observed by the doses of 1.0 and 10 mg Zn/100 g for 15 d. This suggests that high zinc does not inhibit calcification related to the

FIG. 8. Changes of the Concentrations of Calcium and Inorganic Phosphorus, and the Activity of Alkaline Phosphatase in the Serum of Rats orally Administered Zinc Sulfate for 3, 7, 15 and 30 d

The Rats were killed 24 h after the last administration of zinc sulfate (0.1, 1.0 and 10 mg Zn/100 g). Each point is the mean of six animals. Vertical lines represent the S.E.M. Key: a) p < 0.01, compared with the control; (- ▲ - ) 0.1 mg Zn/100 g; (- - - ) 1.0 mg Zn/100 g; (- - - ) 10 mg Zn/100 g.
enzyme in the bone cells. On the contrary, the decrease of calcium level in the serum may bring a reduction of deposition of calcium from the serum into the bone matrix of rats. Thus, the mechanisms of bone resorption by prolonged administration of zinc is complex.

In conclusion, the present study demonstrates that the administration of comparatively low zinc to weaning rats may stimulate the bone growth and calcification at early stage, while prolonged administration of zinc may inhibit these developments. A role of zinc in growth and calcification of bone has been suggested by using zinc-deficient animals\textsuperscript{2-4} although the mechanism has not been clarified. The present finding, that zinc increased DNA content in bone tissue of weanling rats, suggests that the metal plays an important role in the physiological function of bone cells.

REFERENCES