Bioavailability of Zinc Yeast in Rats: Stimulatory Effect on Bone Calcification *in Vivo*

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The bioavailability of zinc yeast as a functional food ingredient in rats was investigated. Zinc yeast, zinc sulfate, or zinc oxide was used. Serum zinc concentrations were significantly increased by a single oral administration of zinc yeast, zinc sulfate or zinc oxide at a dose of 10 mg Zn/100 g body weight. A significant increase was observed 1 hr after administration and it was also seen with the lowest dose of Zn 0.5 mg/100 g of zinc yeast or zinc oxide. A single oral administration of zinc yeast, zinc sulfate, or zinc oxide (10 mg Zn/100 g) caused a significant increase in liver zinc content. A significant increase in femoral-diaphyseal and -metaphyseal zinc contents was observed with the administration of zinc yeast or zinc sulfate. When zinc yeast or zinc oxide (10 mg Zn/100 g) was orally administered once daily for 7 days to rats, a significant increase in zinc levels in the serum, liver, and femoral-metaphyseal tissues was seen. Femoral-diaphyseal zinc content was significantly increased with the administration of zinc yeast. A significant increase in calcium content and alkaline phosphatase activity in the femoral-diaphyseal and -metaphyseal tissues occurred with the administration of zinc yeast or zinc yeast or zinc oxide. The effect of zinc yeast was greater than that of zinc oxide. This study demonstrated that zinc yeast has high bioavailability in rats, and that its administration induces an anabolic effect on bone calcification *in vivo*. Zinc yeast may be useful as a functional food ingredient.

Key words — zinc, zinc yeast, zinc oxide, bone, osteoporosis, bioavailability

INTRODUCTION

Osteoporosis is widely recognized as a major public health problem.¹⁻⁴⁾ Bone loss with increasing age induces osteoporosis. Bone loss may be due to increased bone resorption and decreased bone formation. A decrease in bone mass leads to bone fracture. Pharmacologic and nutritional factors may help to prevent bone loss with increasing age.⁵⁾ Recent studies have shown that zinc,^{6–8)} an essential trace element, isoflavones (including genistein and daidzein)9-11) that occur in large quantities in soybean, and menaquinone-7,12-14) an analogue of vitanmin K_2 which is essential for the γ -carboxylation of the osteocalcin of bone matrix protein, in fermented soybean have a preventive effect on bone loss. These factors have a stimulatory effect on osteoblastic bone formation and an inhibitory effect

on osteoclastic bone resoroption, thereby increasing bone mass.^{15–17)}

Zinc has been demonstrated to have a wide variety of roles in the mammalian system and is essential for growth in humans and many animals.^{18,19)} Bone growth retardation is a common finding in various conditions associated with zinc deficiency, suggesting a nutritional and physiologic significance.²⁰⁾ The cellular mechanism of zinc action has been demonstrated to be stimulation of proliferation and differentiation in osteoblastic cells.^{21,22)} Zinc can stimulate protein synthesis in osteoblastic cells²³⁾ and has been shown to inhibit the formation of osteoclastic cells from bone marrow cells,^{7,24)} indicating that the metal has an inhibitory effect on bone resorption. Thus zinc may play a role in the preservation of bone mass by stimulating bone formation and inhibiting bone resorption.^{8,25)}

The supplemental intake of dietary zinc may have a role in the prevention of osteoporosis with increasing age. Zinc yeast may be useful as a functional food ingredient. However, the bioaviailability of zinc yeast has not been fully clarified. This study was undertaken to determine the bioavialability of

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zinc yeast in rats. We found that the oral administration of zinc yeast in rats causes a significant increase in serum zinc concentration and liver and femoral zinc contents and that the administration has an anabolic effect on bone calcification *in vivo*.

MATERIALS AND METHODS

Chemicals — Zinc oxide and zinc yeast were supplied from Bausch&Lomb Co. Ltd. (Rochester, NY, U.S.A.). Zinc sulfate and other chemicals were reagent grade from Wako Pure Chemical Industries (Osaka, Japan). All water used was glass distilled. Animals — Male Wistar rats (conventional) weighing 90–100 g (4 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). The animals were fed commercial laboratory chow (solid) containing 1.1% calcium and 1.1% phosphorus at a room temperature of 25°C, with free access to distilled water.

Administration Procedures — The water suspension (0.5, 1, 5, and 10 mg Zn/ml/100 g body weight) of the powder of zinc oxide or zinc yeast (10% zinc content) was orally administered to rats through a stomach tube once daily for 7 days. Zinc sulfate (1, 5, and 10 mg Zn/ml/100 g body weight), which was dissolved in distilled water, was orally administered to rats for 7 days. Control rats received distilled water (1.0 ml/100 g body weight) orally. The animals were killed by cardiac puncture under light ether anesthesia, and the blood, liver and femur were removed immediately.

Analytical Procedures — Blood samples were centrifuged for 30 min after collection, and the serum was separated and analyzed immediately. Serum zinc, calcium, and inorganic phosphorus concentrations were determined using an assay kit (Wako Pure Chemical Industries).²⁶⁾

The livers were perfused with ice-cold 0.25 M sucrose solution after exsanguination. The femurs were removed immediately and soaked in ice-cold 0.25 M sucrose solution. The femur was cleaned of soft tissue and marrow, and the diaphyssis and metaphysis (not containing epiphyseal tissue) were separated. The liver tissues and the diaphyseal and metaphyseal tissues were weighed and then digested with nitric acid, dried, and dissolved in 1 N hydrochloride solution. Zinc levels were determined using an assay kit (Wako Pure Chemical Industries). Zinc contents in the liver and bone tissues were expressed as micrograms per gram of wet tissue.

2400 Zinc yeast Zinc sulfate Zinc oxide 1800 Zinc (Jug/dl) 1200 600 0 01 01 3 24 24 0 1 3 6 24 6 Time after administration (hr)

Fig. 1. Change in Serum Zinc Concentration of Rats after a Single Oral Administration of Zinc Compounds *in Vivo* Rats received a single oral administration of zinc yeast, zinc sulfate, or zinc oxide, and 1, 3, 6, and 24 hr later the animals were killed. The dose of zinc was 10 mg/100 g body weight. Each value is the mean \pm S.E.M. of five rats. *p < 0.01 compared with the control (none) value.

To measure femoral calcium content, the diaphyseal and metaphyseal tissues were dried for 16 hr at 110°C, weighed, and then digested with nitric acid.²⁷⁾ Calcium levels were determined using an assay kit. Calcium content in bone tissues was expressed as milligrams per gram of dry bone.

To assay alkaline phosphatase activity, the metaphyseal tissues were immersed in 3.0 ml of ice-cold 6.6 mM barbital buffer (pH 7.4), cut into small pieces, and disrupted for 60 sec with an ultrasonic device. The supernatant, centrifuged at $600 \times g$ for 5 min, was used to measure enzyme activity. Enzyme assay was carried out under optimal conditions. Alkaline phosphatase activity was determined using the method of Walter and Schutt.²⁸⁾ Enzyme activity was expressed as micromol of *p*-nitrophenol liberated per minute per milligram of protein. Protein concentration was determined by the method of Lowry *et al.*²⁹⁾

Statistical Analysis — The significance of difference between values was estimated using Student's *t*-test. *p*-Values of less than 0.05 were considered to indicate statistically significant differences.

RESULTS

Change in Serum Zinc Concentrations in Rats after a Single Oral Administration of Zinc Compounds

The change in zinc concentrations in the serum of rats administered zinc is shown in Fig. 1. Rats received a single oral administration of zinc yeast,



Fig. 2. Effect of Increasing Doses on Serum Zinc Concentration in Rats 3 hr after a Single Oral Administration of Zinc Compounds *in Vivo*

Rats received a single oral administration of zinc yeast, zinc sulfate, or zinc oxide, and 3 hr later the animals were killed. The dose of zinc was 1, 5, or 10 mg/100 g body weight. Each value is the mean \pm S.E.M. of five rats. **p* < 0.01 compared with the control (none) value.

zinc sulfate, or zinc oxide (10 mg Zn/100 g body weight), and the animals were killed at 1, 3, 6, and 24 hr after administration. Serum zinc concentration was significantly increased by the administration of zinc compounds. The maximum increase in serum zinc concentration was seen 6 hr after the administration of zinc yeast or zinc sulfate and 1 hr after the administration of zinc oxide. A significant increase in serum zinc concentration was also seen 24 hr after the administration of the three zinc compounds.

The effect of increasing doses of zinc compounds on serum zinc concentration in rats is shown in Fig. 2. Rats received a single oral administration of zinc yeast, zinc sulfate, or zinc oxide (1, 5, or 10 mg/ 100 g body weight), and 3 hr later the animals were killed. A significant increase in serum zinc concentration was observed with the dose of Zn 1 mg/ 100 g body weight of zinc yeast, zinc sulfate, or zinc oxide. When rats were killed at 1 hr after a single oral administration of zinc yeast or zinc oxide, a significant in serum zinc concentration was observed with the dose of 0.5 mg Zn/100 g body weight of zinc yeast or zinc oxide (Fig. 3).

The change in zinc contents in the liver and femoral tissues of rats after the administration of zinc compounds is shown in Figs. 4 and 5. Rats received a single oral administration of zinc yeast, zinc sulfate, or zinc oxide, and 6 hr later the animals were killed. Liver zinc content was significantly increased with the administration of the three zinc compounds (Fig. 4). Femoral-diaphyseal zinc content was significantly increased with the administration of zinc yeast or zinc sulfate, while the administration of zinc



Fig. 3. Effect of Increasing Doses on Serum Zinc Concentration in Rats 1 hr after a Single Oral Administration of Zinc Yeast or Zinc Oxide *in Vivo*

Rats received a single oral administration of zinc yeast or zinc oxide (0.5, 1, or 10 mg Zn/100 g body weight), and 1 hr later the animals were killed. Each value is the mean \pm S.E.M. of five rats. *p < 0.01 compared with the control (none) value.





Rats received a single oral administration of zinc yeast, zinc sulfate, or zinc oxide, and 3 hr later the animals were killed. The dose of zinc was 10 mg/100 g body weight. Each value is the mean \pm S.E.M. of five rats. **p* < 0.01 compared with the control (none) value.

oxide did not cause an appreciable elevation (Fig. 5). The administration of zinc yeast, zinc sulfate, or zinc oxide resulted in a significant increase in the metaphyseal zinc content (Fig. 5).

Effect of Administration of Zinc Yeast on Bone Components in Rats *in Vivo*

Zinc yeast or zinc oxide (10 mg Zn/100 g body weight) was orally administered once daily for 7 days in rats, and the animals were killed 24 hr after the last administration. The body weight or serum calcium and inorganic phosphorus concentrations did not change significantly after the administration of zinc yeast or zinc oxide for 7 days, while the serum zinc concentration was increased significantly (Table 1). Liver zinc (Fig. 6) and femoral-metaphyseal zinc (Fig. 7) contents were significantly increased with the administration of zinc yeast or zinc oxide. A significant increase in femoral-diaphyseal zinc content occurred after the administration of zinc yeast, but not after zinc oxide administration (Fig. 7).

The change in calcium content and alkaline phosphatase activity in the femoral tissue of rats administered zinc yeast or zinc oxide (10 mg/100 g body weight) once daily for 7 days is shown in Figs. 8 and 9, respectively. Calcium content (Fig. 8) and alkaline phosphatase activity (Fig. 9) in the femoral-diaphyseal and -metaphyseal tissues were significantly elevated after the administration of zinc yeast or zinc oxide. In the femoral-diaphyseal tissues, the effect of zinc yeast on increasing bone calcium content and alkaline phosphatase activity was greater than that of zinc oxide.



Fig. 5. Change in Zinc Content in the Femoral-Diaphyseal and -Metaphyseal Tissue of Rats after a Single Oral Administration of Zinc Compounds *in Vivo*

Rats received a single oral administration of zinc yeast, zinc sulfate, or zinc oxide, and 3 hr later the animals were killed. The dose of zinc was 10 mg/100 g body weight. Each value is the mean \pm S.E.M. of five rats. **p* < 0.01, compared with the control (none) value.

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DISCUSSION

The bioavailability of zinc yeast in rats has not been clarified fully. This study demonstrates that the serum zinc concentration is markedly and dose dependently increased by a single oral administration of zinc yeast, indicating that zinc in the yeast is absorbed by the intestines. The increase in serum zinc concentration with the dose (10 mg Zn/100 g body weight) of zinc yeast showed the same pattern as that of zinc sulfate. Zinc yeast had high bioavailability in rats. In addition, the serum zinc concentration was significantly increased when a zinc oxide suspension was orally administered to rats. However, the increase in serum zinc concentration with the dose of zinc oxide was lower than that of zinc yeast.

The tissue administration of zinc yeast or zinc sulfate caused a significant increase in zinc content in the liver and femoral-diaphyseal and -metaphyseal tissues of rats, indicating that the increased serum zinc is distributed in organ tissues. The dosedependent distribution of serum zinc with the administration of zinc oxide was marked in the liver, while it was slight in the femoral tissues. Thus the oral administration of zinc yeast, zinc sulfate, or zinc oxide resulted in the different patterns of tissue distribution of serum zinc in rats.

Zinc has been shown to have a stimulatory effect on osteoblastic bone formation and an inhibitory effect on osteoclastic bone resorption,^{6–8)} thereby increasing bone mass. The oral administration of zinc yeast to rats for 7 days caused a significant increase in serum zinc concentration and femoraldiaphyseal and -metaphyseal zinc contents. The administration of zinc yeast induced a significant increase in calcium content and alkaline phosphatase activity in the femoral-diaphyseal and -metaphyseal tissues of rats. Alkaline phosphatase is a marker enzyme of osteoblastic cells, which can stimulate bone forma-

Table 1. Change in Body Weight and Serum Components in Rats Administered Zinc Oxide or Zinc Yeast for 7 days

Treatment	Body Weight	Serum Concentration		
	(g)	Zinc (μ g/dl)	Calcium (mg/dl)	Inorganic phosphorus (mg/dl)
Control	110.3 ± 5.1	148.7 ± 6.5	10.64 ± 0.34	9.21 ± 0.54
Zinc oxide	108.4 ± 2.9	$242.2\pm10.4*$	10.32 ± 0.41	9.18 ± 0.41
Zinc yeast	107.8 ± 3.5	$315.0\pm41.2^*$	10.08 ± 0.29	8.65 ± 0.22

Zinc oxide or zinc yeast (10 mg Zn/100 g body weight) was orally administered once daily for 7 days to rats, and they were killed 24 hr after the last administration. Each value is the mean \pm S.E.M. of five rats. *p < 0.01 compared with the control value.



Fig. 6. Change in Zinc Content in the Liver of Rats Orally Administered of Zinc Oxide or Zinc Yeast for 7 days Rats were orally administered zinc oxide or zinc yeast for 7 days

(10 mg Zn/100 g body weight) once daily for 7 days, and the animals were killed 24 hr after the last administration. Each value is the mean \pm S.E.M. of five rats. **p* < 0.01 compared with the control (none) value.



Fig. 7. Change in Zinc Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of Rats Orally Administered of Zinc Oxide or Zinc Yeast for 7 days

Rats were orally administered zinc oxide or zinc yeast (10 mg Zn/ 100 g body weight) once daily for 7 days, and the animals were killed 24 hr after the last administration. Each value is the mean \pm S.E.M. of five rats. **p* < 0.01 compared with the control (none) value. #*p* < 0.01, compared with the value with zinc oxide.

tion and bone calcification.³⁰⁾ The present findings suggest that the oral administration of zinc yeast has a stimulatory effect on bone formation and bone calcification in rats *in vivo*.

The increase in zinc content in the femoral-diaphyseal tissues with the dose of zinc oxide was slight. However, zinc oxide administration caused a significant increase in calcium content and alkaline phosphatase activity in the femoral-diaphyseal tissue. Zinc is mainly distributed in the bone matrix, and bone cellular zinc levels are increased in the femoral tissues of rats administered zinc com-



Fig. 8. Change in Calcium Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of Rats Orally Administered Zinc Oxide or Zinc Yeast for 7 days

Rats were orally administered zinc oxide or zinc yeast (10 mg Zn/ 100 g body weight) once daily for 7 days, and the animals were killed 24 hr after the last administration. Each value is the mean \pm S.E.M. of five rats. **p* < 0.01 compared with the control (none) value. #*p* < 0.01, compared with the value from zinc oxide.





Rats were orally administered zinc oxide or zinc yeast (10 mg Zn/ 100 g body weight) once daily for 7 days, and the animals were killed 24 hr after the last administration. Each value is the mean \pm S.E.M. of five rats. **p* < 0.01 compared with the control (none) value. #*p* < 0.01 compared with the value with zinc oxide.

pounds.^{31,32)} Presumably, zinc is dose dependently elevated in osteoblastic cells of the femoral-diaphyseal tissues of rats with the administration of zinc oxide, thereby stimulating bone calcification.

Zinc deficiency is observed often in humans. The supplemental intake of dietary zinc may be able to alleviate zinc deficiency. Zinc yeast has high bioavilability in rats. The oral administration of zinc yeast to rats induces an anabolic effect on bone components, suggesting that the intake of zinc yeast can prevent bone loss in zinc deficiency. Zinc yeast may be useful as a functional food ingredient in the prevention of osteoporosis in humans.

In conclusion, it has been demonstrated that the oral administration of zinc yeast causes a significant increase in zinc levels in the serum, liver, and femoral tissues of rats *in vivo* and that the administration induces a stimulatory effect on bone calcification *in vivo*. Zinc yeast has high bioavailability in rats.

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