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Adding Zinc Reduces Bone Strength of Rats Fed a Low-Calcium Diet

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ABSTRACT

This experiment examined skeletal effects of moderate zinc (Zn) supplementation of a low-calcium diet. Male weanling rats were fed experimental diets for about 4 wk. One diet was adequate (control), whereas two others were calcium-deficient, but otherwise adequate. One of the low-calcium (Ca) diets was supplemented with Zn. Dimensions, weight, mineral content, and mechanical properties of femurs were measured. Ca deficiency reduced bone mineral content and strength markedly. Adding Zn to the low-Ca diet further reduced bone strength and elasticity, compared with the unsupplemented low-Ca diet. When the Ca intake is low, possible benefits of Zn supplements should be weighed against risk of deterioration of mechanical properties of bone.

Index Entries: Zinc; calcium; bone; mechanical properties; strength.

INTRODUCTION

Zinc (Zn) has come to be widely used as a nutritional supplement *(1)* to correct possible deficiencies and for various percieved benefits *(1–5)*; however, Zn supplementation at high levels is not without risk. Supplemental Zn sometimes has reduced high density lipoprotein (HDL) cholesterol *(6–8)*, impaired some immunological responses *(5,9)*, and adversely affected Ca metabolism *(10–13)*. Some effects may be indirect consequences of deficiencies of copper (Cu) or iron (Fe) induced by large intakes of Zn *(7, 9, 14)*. Compared with effects of a large excess of

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Zn, less is known about consequences of moderate Zn supplements, in the range of 15–100 mg/d for human adults *(9)*.

As nutrients, Zn and Ca are interdependent. A large daily intake of Zn (140 mg) reduced men's absorption of Ca from a diet providing about 30% of recommended Ca, but had no such effect when the diet was adequate in Ca *(11)*. Although the considerable amount of Zn found in bone is slowly released *(15)*, feeding a low-Ca diet caused mobilization of Zn from bone and increased its availability for other tissues *(16)*. Alkaline phosphatase activity of bone was sensitive to lowered Zn state *(17)*. Synthesis of organic, as well as inorganic, bone phase was reduced in Zn deficiency *(18,19)*, which interfered with maturation and mineralization of bone *(20)* and altered total bone Ca content *(13)*. Excessive Zn slowed growth and limited size of hydroxyapatite crystals *(10)*, while increasing bone resorption *(21)*. Therefore, the requirement or tolerance for Zn may depend on Ca status *(12,22)*. These findings together suggest that ingestion of supplemental Zn without adequate dietary Ca may be harmful. Women and elderly persons (who are at risk of excessive Ca loss) are likely to need Zn supplements or to take them *(2–5)*. Further information about possible effects of Zn status on the skeleton is needed.

This study was undertaken to determine if, in Ca-depleted rats, addition of a moderate amount of Zn to a Zn-adequate diet adversely affects bone.

PROCEDURES

Young male Sprague-Dawley rats** weighing 81 ± 5 (SD) g, were divided into three groups of 12–14 animals each. Following the protocol approved by the local animal care oversight committee, rats were housed individually in stainless steel, wire-mesh cages in an air-conditioned laboratory with automatic 12-h lighting cycle. Powdered food and distilled water were given ad libitum and replenished daily. Animals were weighed three times each week and examined daily for abnormalities.

Diets were designed to meet standards then set for nutrients *(23)*, except for experimental variables. The diets contained, in percent by weight: vitamin test casein, 21.5; DL-methionine, 0.3; fat,† 19.0; cornstarch, 27.0; sucrose, 16.0; Alphacel cellulose, 11.6; AIN-76™ or modified mineral mix† sucrose, 3.5; AIN-76 vitamin mix *(23)*, 1.0; choline chloride, 0.2; menadione, 0.0001. The control diet was adequate in both Ca and Zn. Mineral mixes for the other groups were formulated to provide 30%

**Hormone Assay Laboratories, Chicago, IL.

†The fat was 1:1:1 mixture of olive oil, partly hydrogenated vegetable shortening, and beef tallow having a P:S ratio (by analysis) of 0.5. Sucrose and fats were purchased locally, and other ingredients were obtained from ICN Biochemicals, Inc., Cleveland OH.

of the Ca and 100% of the phosphorus fed to controls, with added Zn (–Ca + Zn diet) or without it (–Ca diet). By analysis, control, –Ca, and –Ca+Zn diets provided, respectively, 4630, 1400, and 1460 mg Ca and 44, 43, and 72 mg Zn/kg diet, compared with recommended levels of 5200 mg Ca and 30 mg Zn/kg *(23)*.

Purified diets were fed for 25, 27, or 29 d. Animals were fasted overnight before being killed. Sodium pentobarbital solution was administered ip, and blood was removed from the abdominal aorta. Blood was transferred to heparinized tubes having Zn-free stoppers until plasma could be separated for storage and analysis. Femurs were removed and cleaned manually of adhering tissue. Each femur was weighed, wrapped in saline-soaked gauze, and sealed in plastic for storage at –10°C.

After equilibration at room temperature, both femurs from each rat were individually broken in a three-point flexure test *(24)*. From load-deformation tracings and internal and external diameters at the break point of the bone, the load and stress at breaking and the modulus of elasticity were determined. Each broken femur was dried, weighted, and analyzed separately. Dry bone was ashed at 600°C. Ca determinations used ash or plasma in dilute hydrochloric acid containing 0.1% lanthanum. Plasma was diluted 1:5 with deionized water to measure Zn; standard solutions contained 5% glycerol to mimic the viscosity of plasma solutions. Zn and Ca were measured by atomic absorption spectrometry.

Data were analyzed by the SAS General Linear Models (GLM) procedure *(25)*. The model included dietary treatment group and day of killing or testing. The GLM Repeated Measures option was used for paired bones. Least-squares (adjusted) treatment means were compared by *t*-tests for preselected pairs of groups to test for hypothesized negative effects of Ca deficiency, positive effects of Zn on growth and plasma Zn, and possible adverse effects of Zn on bone strength. Data were transformed logarithmically when appropriate to normalize distribution of values. Differences between treatment groups were considered significant at $P \leq 0.05$.

RESULTS

Compared with that for controls, weight gain of –Ca group was slower, and less food was consumed (Table 1), but efficiency of food utilization was not affected by Ca deficiency. Weight gain of –Ca + Zn exceeded that of –Ca animals (Table 1), even though the –Ca diet already exceeded the 30 mg Zn/kg diet recommended for the rat *(20)*. Neither of the small increases in mean food intake and in food efficiency ratio was significant or could alone account for the difference in body weight gain.

Femurs were the same average length (33.6 ± 0.2 [SE] mm) in all groups, but –Ca and –Ca + Zn femurs weighed 27–28% less and showed 25–28% less cross-sectional bone area than those of controls (Table 1). Both of the low-Ca groups had a 40% reduction in total Ca and ash in femurs

Table 1
Growth and Femur and Plasma Composition of Rats Subjected to Ca Deficiency, with or Without Supplemental Zn

Diet group	Wt gain, g/wk*	Food intake, g/wk*	Food efficiency, g gain / g food	Femur				Plasma
				Dry wt, mg	Cross-section, mm^2	Ash, mg/g dry bone	Zn, μg/g ash	Zn, mg/L
Control	70	120	0.603	427	6.59	567	410	1.35
-Ca	64[a]	109[a]	0.601	306[a]	4.72[a]	455[a]	514[a]	1.49[a]
-Ca+Zn	68[b]	112	0.618	311	4.93	451	564[b]	1.67[b]
SE	1.5	2.5	0.008	6.1	0.17	2.9	12	0.05

*Average based on three consecutive weekly measurements for each rat.
Superscripts indicate significant differences ($P \leq 0.05$): [a]control vs –Ca; [b]–Ca vs –Ca + Zn.

(data not shown). The magnitude of these effects of Ca deficiency on femur considerably exceeded the 9% decrease in body weight gain.

The smaller femurs from –Ca rats contained 72 μg total Zn, 28% less than the 99 μg in those of controls (calculated from data in Table 1), consistent with the extent of loss of total bone mass and resulting in an increased Zn:Ca ratio in bone. Zn supplementation increased the Zn concentration in bone ash (Table 1), although the total femur Zn, 79 μg, was not restored to values of controls. Plasma Zn was elevated in both of the low-Ca groups, with higher concentrations in –Ca + Zn than in –Ca rats. Thus, Ca deficiency extensively reduced calcification of bone, with an apparent shift of some bone zinc into the plasma compartment. Increasing the Zn intake raised both femur and plasma Zn concentrations.

Inadequate bone mineralization was a primary effect of Ca deficiency. The decrease in ash/g dry bone of the –Ca group (Table 1) demonstrates that mineral loss exceeded the reduction in organic components of bone. The mass of organic matter, as might be estimated from dry weight minus ash, appears to have been affected less by diets than the mineral component. The reduction in mineral resulting from Ca deficiency was accompanied by increased Zn concentration in femur ash, according to amount of Zn fed.

Along with the lower mineral content of bone in Ca deficient animals, bone strength (breaking load and stress) and elasticity were dimin-

Table 2
Mechanical Properties* of Femurs from Rats Fed Ca-Deficient Diets with or Without Added Zn, and from Controls

Diet group	Breaking load, kg	Breaking stress, MPa	Modulus of elasticity, MPa
Control	7.95 (7.58-8.33)	78.8 (73.9-84.2)	4950 (4480-5470)
-Ca	3.46[a] (3.33-3.63)	46.3[a] (43.3-49.4)	2850[a] (2570-3150)
-Ca+Zn	3.12[b] (2.97-3.28)	38.8[b] (36.3-41.6)	2220[b] (2000-2470)

*Analyzed as log-transformed values; range in parentheses is mean ± SE.
Superscripts indicate significant differences ($P \leq 0.05$): [a]control vs –Ca; [b]–Ca vs –Ca + Zn.

ished (Table 2). The –Ca bones broke under less than half the load required to break control bones. Although the addition of Zn to the low-Ca diet did not affect total ash content of femur (Table 1), the low breaking load for the –Ca group was further reduced when Zn was added in the –Ca + Zn diet (Table 2). Zn supplementation also significantly decreased the breaking stress (load adjusted for bone size) and the modulus of elasticity (stiffness) of the femur.

DISCUSSION

Ca deficiency reduced the ash content of bone and, consequently, reduced the load the bone could support, despite the modeling of growing bone to compensate efficiently for increasing load *(26)*. Length and outside diameters of femurs were not reduced by Ca depletion, although dry weight and cross-sectional bone area indicated extensive reduction in bone mass (Table 1). Decreased load tolerance reflected reduced functional capacity of the whole bone, whereas low stress and elasticity values indicated deterioration in quality of bone material. Data presented here confirm the uncertainty of reliance solely on bone density data *(27)* to predict change in mechanical behavior of bone. All the changes in mechanical properties resulting from Ca deficiency were exacerbated by addition of Zn to the low-Ca diet, without further decrease in ash concentration. Thus, the detrimental effect of Zn could not be explained by loss of bone mass, but indicated qualitative changes in bone.

Zn is necessary to produce and maintain organic components of bone matrix *(18–19)* as well as for normal calcification *(12,20)*. On the other hand, too high a concentration of Zn can interfere with hydroxyapatite crystal growth *(10)* and accelerate bone resorption *(13)*. Varying the dietary Zn content also can affect bone metabolism by altering membrane properties *(24)*, DNA synthesis, or gene expression *(3,17,28)*, by participation in hormone–receptor interactions *(14,28)*, and by regulating activities of Zn-dependent enzymes (such as alkaline phosphatase) *(13,17)*. Added Zn in this study did not significantly affect bone mass or ash content in Ca-deficient animals. Supplemental Zn did, however, reduce the strength of the whole femur (load borne), and the strength (stress) and stiffness (elasticity) of the bone material. These properties are dependent on both the mineral and organic phases of bone and on their interaction *(26)*. Since Zn has roles in forming or regulating both phases, it is not possible to identify which action was responsible for reduced strength of bone at the high-Zn level.

Although direct actions of Zn may explain changes in bone properties, other factors may be involved. High Zn intake can produce Cu deficiency *(3,28)*. Decreased Cu-dependent lysyl oxylase activity and collagen crosslinking would reduce tensile strength of organic bone matrix. We did not assess Cu status, but such effects have been reported mainly above a dietary Zn:Cu ratio of about 20–30 *(6–8,21)*. In this experiment, the highest Zn:Cu ratio was about 12. It is not known if Ca deficiency alters the quantitative interactions between Zn and Cu.

Further work is needed to clarify which of several possible mechanisms are responsible for the effects of Zn supplementation on femur properties. Under what other dietary conditions Zn may exert similar effects remains to be determined.

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