Long-Term Marginal Zinc Supply Is Not Detrimental to the Skeleton of Aged Female Rats

Reinhold G. Erben, Katja Lausmann, Paul Roschger, Christiane Schüler, Monika Skalicky, Klaus Klaushofer, and Wilhelm Windisch

Abstract

In this experiment, we investigated the long-term effects of a marginal zinc (Zn) supply on bone metabolism in aged rats. Nine-mo-old female Fischer-344 rats were divided into 8 weight-matched groups of 8 rats each. All rats were adapted for 1 mo to restrictive feeding (7.5 g/d) of a purified diet containing 8 g/kg sodium phytate and 64 mg/kg Zn. Control rats were pair-fed throughout the experiment. During the 1-mo depletion phase, controls received the Zn-replete diet with 64 mg/kg Zn, whereas Zn-deficient rats were fed the same diet with 2.2 mg/kg Zn. The depletion phase was followed by a 3-mo marginal phase in which the rats fed the diet with 2.2 mg/kg Zn received an additional daily Zn supplement of 75 μg Zn/rat by gavage. In the following 2-mo repletion phase, a marginal group was switched to the Zn-replete diet, while the other groups were maintained on marginal Zn supply or on the Zn-replete diet. Zn depletion and marginal Zn reduced serum and bone Zn and serum alkaline phosphatase activity. Zn repletion normalized serum Zn. However, apart from subtle changes in bone mineralization density distribution, Zn deficiency was not associated with detrimental effects on bone mineral density, turnover, architecture, or biomechanics relative to control rats at any time point. Our data suggest that Zn does not play an essential role in bone metabolism in aged rats and cast doubt on the hypothesis that Zn deficiency is a risk factor for osteoporosis. J. Nutr. 139: 703–709, 2009.

Introduction

Although firm clinical evidence is lacking, zinc (Zn) deficiency has been implicated in playing a role as a risk factor in the development of human osteoporosis for many years (1–4). The Zn concentration in bone is higher than in most other tissues (5), Zn accumulates in newly formed bone (6–8), and Zn concentrations in bone respond readily to Zn depletion (9–11). Furthermore, the micronutrient Zn is an essential cofactor for several Zn-dependent enzymes in bone (12,13) and Zn deficiency has been shown to impair collagen biosynthesis (14–17). In addition, administration of a Zn-chelating dipeptide stimulates bone protein synthesis in vitro (18,19). Therefore, Zn may be involved in bone mineralization and in osteoblastic matrix synthesis. Moreover, in vitro studies have shown that Zn may also have antiresorptive effects on mature osteoclasts or osteoclast precursors (20–23).

However, most of the evidence supporting the notion that Zn may play a role in human adult bone homeostasis comes from experimental studies in growing animals or from in vitro studies. There is a large body of literature showing that Zn deficiency is associated with osteopenia, impaired bone mineralization, and reduced biomechanical properties of bone in growing rodents, pigs, and monkeys (24–28). However, the role of acquired adult Zn deficiency in skeletal homeostasis is still unclear. Studies in skeletally mature, nongrowing rats addressing the problem of acquired adult Zn deficiency are lacking, mainly because nongrowing rats cannot be made Zn deficient by feeding a Zn-deficient diet. The large body stores of Zn and the reduced daily requirements protect adult rats fed Zn-deficient diets for many months from overt Zn deficiency. However, Windisch and Kirchgessner (29) conceived a method to accomplish Zn depletion in adult rats within a short period of time. This method is based on the idea that feeding a Zn-deficient diet enriched with phytate causes high fecal losses of Zn through inhibition of gastrointestinal reuptake of the large amount of Zn secreted daily into the gut via digestive secretions. Phytate strongly binds divalent cations and cannot be enzymatically cleaved by mono-gastric animals. Therefore, phytate forms insoluble complexes...
with Zn and other divalent ions that cannot be absorbed in the intestine.

Our experiment uses this method to elucidate further the role of Zn in bone metabolism of aged, 9-mo-old female rats. The term “aged” in this manuscript refers to nongrowing or slowly growing rat models used in bone research (30–32). The aim of this study was to test the hypothesis that long-term acquired Zn deficiency would result in osteopenia in aged rats. An important confounder for this type of study is that Zn deficiency has profound effects on appetite regulation (33,34). Therefore, a long-term study necessary to evaluate the role of Zn in the pathogenesis of osteoporosis is not feasible in Zn-deficient animals characterized by a sustained negative energy balance due to the secondary sequela of inanition. Therefore, this study was planned as a 3-phase experiment: a 1-mo Zn depletion phase was followed by a 3-mo marginal Zn supply phase, in which the rats were given a Zn supply just high enough to keep the rats in energetic steady state, and in a final 2-mo repletion phase some marginal-Zn rats were returned to receiving the Zn-replete diet.

Materials and Methods

Animal procedures. All animal procedures were approved by the Ethical Committee of the Ludwig Maximilians University, Munich, Germany, and the Bavarian government authorities. Sixty-four 9-mo-old female Fischer-344 rats were divided into 8 weight-matched groups of 8 rats each (study design shown in Fig. 1). At the beginning of the experiment, all rats were adapted for 1 mo to restrictive feeding (7.5 g/d) of a purified diet (35) adjusted to a total Zn concentration of 64 mg/kg by addition of 264 mg/kg ZnSO₄·7 H₂O. The daily amount of diet necessary for restrictive feeding was calculated on the basis of the metabolizable energy of the purified diet (18 kJ/g) and the maintenance requirements of an adult 200-g rat (~135 kJ) (0.45 MJ/kg⁰.⁷⁵). The Zn-deficient purified diet was fortified with 8 g sodium phytate/kg (dodecasodium phytate; Sigma-Aldrich) and contained 2.2 mg/kg Zn as determined by atomic absorption spectrophotometry (AAS)⁷ (composition of diets shown in Table 1). Rats consumed ad libitum deionized water. Control rats were pair-fed on a group mean basis throughout the experiment. During the 1-mo depletion phase, controls received the Zn-replete diet with 64 mg/kg Zn, whereas Zn-deficient rats were fed the same diet with 2.2 mg/kg Zn. Both diets (control and Zn deficient) were balanced with respect to equal and sufficient amounts of minerals except for Zn (Ca, 0.65%; nonphytate P, 0.45%; Mg, 0.10%; K, 0.60%; Na, 0.25%; Cl, 0.37%; Fe, 100 mg/kg; Mn, 100 mg/kg; Cu, 30 mg/kg). The depletion phase was followed by a 3-mo marginal phase in which the rats on the diet with 2.2 mg/kg Zn received an additional daily Zn supplement of 75 mg Zn per rat as ZnSO₄ (Sigma) dissolved in bi-distilled water by gavage. Control animals received bi-distilled water by gavage. At the beginning of the marginal phase, the amount of Zn given via gavage was increased in a stepwise manner until food consumption reached baseline values. We increased the daily amount of Zn given via gavage in increments of 5–10 μg Zn/rat every other day until the mean daily food consumption reached 7–7.5 g/rat. The time necessary for this adaptation period, ~4 wk, was part of the 3-mo marginal phase. We found that an oral Zn supplement of 75 μg Zn/normolzation and fed diet was not sufficient food consumption. To rule out that this dose of supplementary Zn would enable the marginal Zn rats to refill their Zn body stores, we reduced the oral Zn supplements every 2 wk for 1 d to 65 μg Zn/rat. Food intake promptly decreased the next day in all rats, showing that the marginal Zn rats were kept for months on a labile equilibrium not allowing them to refill their Zn body stores. In the following 2-mo repletion phase, a marginal group was switched to the Zn-replete diet, while the other groups were maintained on marginal Zn supply or on the Zn-replete diet. Groups of ~7–8 rats each were killed at baseline, after the depletion phase, and after the marginal phase by exsanguination from the abdominal aorta under ketamine/xylazine (50/10 mg/kg) anesthesia. During the repletion phase, 6 rats had to be killed because of an infection with the dermatophyte Microsporum canis. Therefore, group size was only ~4–7 rats at the end of the experiment. Calcium (Sigma-Aldrich) at a dose of 20 mg/kg body weight was injected subcutaneously on the 9th and 4th d before necropsy. Urine was collected in metabolic cages during a 14-h period overnight prior to necropsy. During necropsy, the right ureter horn was removed from each animal, rinsed in physiological saline, blotted dry, and weighed. Bones were processed as described below.

Blood and urine analysis. Total calcium, sodium, and potassium in serum and urine were determined by flame photometry (EFOX 5053, Eppendorf). Serum alkaline phosphate (ALP) activity, creatine kinase activity, aspartate aminotransferase, urea, creatinine, albumin, total bilirubin, and phosphorus as well as urinary creatine and urinary

![FIGURE 1](https://example.com/figure1.png)

**FIGURE 1** Experimental design. Sixty-four 9-mo-old female Fischer 344 rats (n = 8 per group) were adapted to a purified diet containing 64 mg/kg Zn and 8 g/kg sodium phytate for 1 mo. Thereafter, some groups received the Zn deficient diet containing 2 mg/kg Zn and 8 g/kg sodium phytate for 1 mo. With the exception of Zn concentration, control and Zn deficient diet were identical. The depletion phase was followed by a 3-mo marginal Zn phase, in which the rats on the Zn deficient diet received an additional daily Zn supplement via gavage just high enough to keep the rats in energetic steady state (75 μg/d Zn). Finally, marginal Zn rats were switched back to the control diet during a 2-mo repletion period. Vertical arrows denote necropsies.

**TABLE 1** Composition of the diets

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<tr>
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<th>Zn-replete control diet</th>
<th>Zn-deficient diet</th>
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<tr>
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<td>2</td>
</tr>
<tr>
<td>Na, g/kg</td>
<td>8.0</td>
<td>8.0</td>
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<tr>
<td>Protein</td>
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<tr>
<td>Corn starch, g/kg</td>
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<td>411.6</td>
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<td>Sucrose, g/kg</td>
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<td>280.0</td>
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<td>Cellulose, g/kg</td>
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</tr>
<tr>
<td>Sunflower oil, g/kg</td>
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<td>10.0</td>
</tr>
<tr>
<td>Coconut fat, g/kg</td>
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<td>75.0</td>
</tr>
<tr>
<td>t-Methionine, g/kg</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamins, g/kg</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Minerals, g/kg</td>
<td>45.9</td>
<td>45.9</td>
</tr>
<tr>
<td>ZnSO₄·7 H₂O, mg/l</td>
<td>0.264</td>
<td>0</td>
</tr>
</tbody>
</table>

1 The Zn concentration in the diets was analyzed postmanufacture by AAS.
2 Per kg diet: 1.5 mg all-trans retinol, 26 μg cholecalciferol, 150 mg α-tocopherolacetate, 5 mg menadione-Na-bisulphite, 5 mg thiaminmononitrate, 10 mg riboflavin, 6 mg pyridoxine hydrochloride, 20 mg Ca-pantothenate, 50 mg nicotinic acid, 1000 mg choline chloride, 1000 μg folic acid, 200 μg biotin, 25 μg cyanocobalamine.
3 Per kg diet: 2.00 g Na₂HPO₄·2 H₂O, 17.6 g KH₂PO₄, 1.73 g KCl, 16.2 g CaCO₃, 8.4 g MgCl₂·6 H₂O, 0.496 g FeSO₄·7 H₂O, 0.117 g CuSO₄·5 H₂O, 0.308 g MnSO₄·H₂O, 2.62 mg KI, 1.20 mg NaF, 4.48 mg Na₂S·7H₂O, 0.504 mg Na₂MoO₄·2 H₂O, 0.887 mg NaSeO₃, 6 H₂O, 0.513 mg CrCl₃·6 H₂O.

⁷ Abbreviations used: AAS, atomic absorption spectrophotometry; ALP, alkaline phosphatase; BMD, bone mineral density; BMDD, bone mineralization density distribution; Ca₁₂₅MEAN, weighted mean Ca concentration; Ca₂₀MEAN, most frequent Ca concentration; CaWidth, width of the distribution; DPD, deoxyxypyrinidol; qBEI, quantitative backscattered electron imaging; pQCT, peripheral quantitative computed tomography.
phosphorus were analyzed on a Hitachi 766 Autoanalyzer (Boehringer Mannheim). Total deoxypyridinoline (DPD) in urine, a specific biochemical marker of bone resorption (36), was determined after acid hydrolysis by ELISA (Total DPD, Metra Biosystems).

**Zn analyses.** The left femora were dried for 3 h at 105°C. Thereafter, the bones were weighed and ashed for 48 h in platinum dishes at 480°C in a muffle furnace. Subsequently, the ash was dissolved in 0.6 mol/L HCl. The Zn concentrations of serum (diluted 1:5 with bi-distilled water) and of the femur solutions were directly determined by AAS (Model 5100, Perkin-Elmer). The Zn concentration in bone was expressed as a ratio to dry weight. The Zn concentrations in the diets were measured postmanufacture by AAS as described (35).

**Bone mineral density measurements.** We measured the volumetric bone mineral density (BMD) of tibias and L4 vertebrae stored in 70% ethanol by peripheral quantitative computed tomography (pQCT) using a XCT Research M+ pQCT machine (Stratec Medizintechnik). One slice in the mid-diaphysis of the tibiae located 2 mm proximal to the tibiofibular junction and 1 slice in the proximal tibial metaphysis located 2 mm distal from the growth plate were measured. In the L4 vertebrae, 3 slices were measured, 1 in a mid-transversal plane and 2 located 1.6 mm rostral and caudal of the mid-transversal plane. BMD values of the L4 vertebral body were calculated as the mean of 3 slices. A voxel size of 0.070 mm and a threshold of 710 mg/cm³ were used for the calculation of cortical BMD.

**Bone mineralization density distribution measurements.** Bone mineralization density distribution (BMDD) was measured on tibias embedded in methylmethacrylate by quantitative backscattered electron imaging (qBEI) as described in detail before (37,38). After polishing (FM15 Logitech) and carbon coating (Argar SEM Carbon coater, Argar Scientific Limited), the samples were analyzed using a digital scanning electron microscope with a 4-quadrant semiconductor backscattered electron detector (DSM 962, C. Zeiss). All measurements were made without knowledge of the treatment groups. For each tibia, 5-6 regions of the same size (2–2.5 mm wide) were scanned in the ephiphyseal spongiosa, metaphyseal spongiosa, and cortical bone. Carbon and aluminum were used for gray level references and osteoid and hydroxyapatite were employed as references to convert gray level values into calcium concentration values. The frequency, given as percentage of bone area, of pixels of a certain calcium (Ca) concentration was used to generate BMDD histograms. From the BMDD histograms, the weighted mean Ca concentration (CaBar), the most frequent Ca concentration (CaPeak), and the width of the distribution (CaWidth) were calculated.

**Cancellous bone histology and histomorphometry.** At necropsy, the proximal right tibia and L1 vertebrae from all rats were defleshed and fixed immediately in 40% ethanol at 4°C for 48 h. After fixation, the bones were embedded undecalcified in methylmethacrylate, as described previously (39). Five-micrometer-thick sections were prepared using a HM 360 microtome (Microm). Quantitative cancellous bone histomorphometry was performed on mid sagittal sections from the proximal tibias and on median sections of the L1 vertebral bodies as described in detail elsewhere (32).

**Bone biomechanics.** L2 vertebrae were defleshed, wrapped in gauze soaked with 0.9% NaCl, and stored frozen at –20°C. Prior to testing, the thawed bones were stored in 0.9% NaCl at room temperature overnight. To fix the vertebrae for trimming, pipette tips were glued upright onto glass slides and the pipette tips were inserted and glued into the spinal canals of the L2 vertebrae. Subsequently, both intervertebral discs and the endplates were removed with the help of a water-cooled diamond precision band saw (Exakt). From each vertebra, a 3.0-mm-high cylinder with plano-parallel ends was prepared. The remaining processi were removed with a fine electric saw (King Craft, Stepper). The vertebral body cylinders were loaded to failure by compression tests using a Zwick Z200/TN2A materials testing machine with a 1-kN force detector and a force resolution of 0.01 N. Crosshead speed during testing was 0.2 mm/min. From the force displacement data, ultimate force (Fmax, N) was calculated.

**Statistical analysis.** Statistics were computed using SPSS for Windows 11.0 and StatView 4.5 (Abacus Concepts). Statistical comparisons between the control and the Zn-deficient groups after the depletion and the marginal Zn phase were made using a 2-sided t test. The 3 groups after the repletion phase were compared by 1-way ANOVA. When the ANOVA performed over all groups indicated a significant (P < 0.05) difference among the groups, statistical differences between 2 groups were subsequently evaluated using a least significant difference test as post hoc test. Comparisons between the baseline group and the other groups at later time points were made by ANOVA followed by Dunnett’s multiple comparison test using the baseline group as the control group. P-values of <0.05 were considered significant. The data are presented as the means ± SEM.

**Results**

**Effects of Zn depletion and marginal Zn phase on food consumption.** For 1 mo before the start of this experiment, all rats were adapted to restrictive feeding (7.5 g/d) of the purified diet. This amount of diet was calculated to result in energetic steady state. During the adaptation phase, body weight remained constant (data not shown). Zn deficiency is associated with loss of appetite (33,34). This effect was also observed in our experiment. Within 1 wk of Zn depletion, the food consumption in Zn-depleted rats fell from 7.5 ± 0.0 g/d to 4.0 ± 1.0 g/d. After 2 wk on the depletion diet, the food consumption was 2.5 ± 0.6 g/d and remained between 1.5 and 3 g until the end of the depletion phase. Food consumption returned to 7–7.5 g/d during the marginal phase with the help of a daily oral Zn supplement of 75 µg Zn/rat administered via gavage in rats fed the Zn-deficient diet. Together with the Zn concentration of the low-Zn diet, the 75 µg Zn/d given via gavage correspond to a diet with 12.2 mg/kg Zn.

**Effects of Zn depletion and marginal Zn supply on body weight, Zn concentrations in serum and bone, and biochemical parameters.** Due to the marked depression in food consumption during the depletion phase, we observed a 7% loss of body weight in the Zn-depleted rats and a 2% loss in the pair-fed controls (Fig. 2A). After the marginal phase, body weight was nonsignificantly reduced in marginal-Zn rats compared with Zn-replete rats (P = 0.067). At all other time points, body weight did not differ between control and Zn-depleted or marginal-Zn rats. Over the whole 7-mo experiment, Zn-replete and marginal-Zn rats lost 8 and 13% of body weight relative to baseline, respectively, showing that all rats remained in slightly negative energy balance throughout the entire study.

Serum Zn decreased in Zn-depleted rats during the depletion phase and was lower in Zn-depleted and marginal-Zn rats than in the Zn-replete controls throughout the study (Fig. 2B). However, we did not observe clinical signs of overt Zn deficiency such as dermal lesions at any time point during the experiment. Dietary repletion of Zn normalized serum Zn. Similarly, Zn depletion reduced bone Zn compared with Zn-replete controls (Fig. 2C). However, femur Zn continued to decrease in marginal-Zn rats throughout the study and did not return to Zn-replete control levels during the repletion phase, showing that marginal-Zn rats continued to deplete bone stores of Zn throughout the experiment and that a 2-mo repletion phase is too short to fully refill Zn stores in the bone compartment after long-term marginal-Zn supply.

ALP is a Zn-dependent enzyme that can be used as a biological indicator for Zn deficiency (40). In agreement with reduced circulating Zn concentrations, serum ALP activity decreased by 44% after Zn depletion and by 28% after the marginal phase compared with control rats (Fig. 2D). However, because ALP activity decreased in Zn-replete controls toward the end of the depletion phase, we did not observe clinical signs of overt Zn deficiency (33,34).
study, serum ALP activity was similar in all groups after the repletion phase. Apart from an age-dependent decline in all groups, urinary DPD excretion normalized to creatinine excretion did not differ between Zn-deficient and Zn-replete rats at any time point of the study (Fig. 2E). Urinary creatinine did not differ between the groups at any time point (data not shown). In addition, Zn depletion or marginal Zn did not induce alterations in mineral homeostasis or in any other chemical parameters in serum and urine analyzed in this study (data not shown).

Zn deficiency may also affect ovarian sex hormone production and uterine responsiveness to estrogen (41,42). Therefore, we examined the uterine weight as a biological read-out for circulating estrogen levels and uterine responsiveness to estrogen in this study. However, the uterine weight was not altered in Zn-depleted or marginal-Zn rats compared with controls at all time points (data not shown).

**FIGURE 2** Body weight (A), serum Zn concentration (B), femur Zn concentration (C), serum ALP (D), and urinary (Ur) DPD/creatinine (DPD/Crea) excretion (E) plotted as a function of time after start of the experiment in Zn-depleted, marginal-Zn, Zn-replete, and control rats. Each data point represents the mean ± SEM, n = 4–8. Symbols indicate significant differences, P < 0.05: * vs. control, # vs. marginal Zn, a vs. baseline.

**FIGURE 3** Trabecular BMD of the proximal tibia (A) and the 4th lumbar vertebral body (B) and total BMD of the tibial shaft (C) measured by pQCT, plotted as a function of time after start of the experiment in Zn-depleted, marginal-Zn, Zn-replete, and control rats. Each data point represents the mean ± SEM, n = 4–8. Symbols indicate significant differences, P < 0.05: a vs. baseline.
Skeletal effects of Zn depletion and of marginal Zn supply.
To evaluate the skeletal effects of a marginal Zn supply in the current long-term experiment, we initially performed pQCT analyses of the tibial shaft, the proximal tibial metaphysis, and the L4 lumbar vertebral body (Fig. 3A–C). To our surprise, Zn depletion or marginal Zn was not associated with altered total or trabecular BMD at any of the sites investigated. The results from the pQCT analyses were fully confirmed by histomor-

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FIGURE 4 Cancellous bone area (B.Ar/T.Ar) in the proximal tibial metaphysis (4A) and the first lumbar vertebral body (B), as well as osteoclast numbers (N.Oc/Md.Pm) (C) and bone formation rate (BFR/ B.Pm) (D) in lumbar vertebral cancellous bone measured by histomorphometry and plotted as a function of time after start of the experiment in Zn-depleted, marginal-Zn, Zn-replete, and control rats. Each data point represents the mean ± SEM, n = 4–8. Symbols indicate significant differences, P < 0.05: * vs. control, * vs. baseline.

FIGURE 5 Maximum load of bone cylinders prepared from 2nd lumbar vertebral bodies loaded to failure in axial compression tests, plotted as a function of time after start of the experiment in Zn-depleted, marginal-Zn, Zn-replete, and control rats. Each data point represents the mean ± SEM, n = 4–8.

Discussion
This study was undertaken to clarify the role of Zn in the maintenance of an adult skeleton. To our knowledge, this is the first study investigating the bony effects of acquired Zn deficiency in aged rats. We defined marginal Zn supply in this study as the lowest Zn supply that results in an adequate intake of dietary energy. We reasoned that a long-term experiment with rats on a sustained negative energy balance would not make sense. Our data clearly showed that long-term marginal Zn supply at a level that was just sufficient for normal appetite and energy intake had no detrimental effects on bone mass, architecture, turnover, or
slightly decreased bone turnover during the whole experimental and Ca PEAK the most frequently occurring Ca concentration.

1 Values are means ± SEM; *Different from Zn-replete control, P < 0.05.

TABLE 2 BMDD measured by qBEI in aged female rats after 3 mo of marginal-Zn supply and in Zn-replete controls

<table>
<thead>
<tr>
<th>Site</th>
<th>Control</th>
<th>Marginal Zn</th>
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<tr>
<td></td>
<td>n</td>
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<tr>
<td>Epiphyseal spongiosa, mmol/g</td>
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<td></td>
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<td>CAMEAN</td>
<td>6.11 ± 0.09</td>
<td>6.24 ± 0.08*</td>
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<tr>
<td>CAFAK</td>
<td>6.30 ± 0.10</td>
<td>6.41 ± 0.06*</td>
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<tr>
<td>CAMWIDTH</td>
<td>0.768 ± 0.048</td>
<td>0.803 ± 0.065</td>
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<td>Metaphyseal spongiosa, mmol/g</td>
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<tr>
<td>CAMEAN</td>
<td>5.99 ± 0.11</td>
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<tr>
<td>CAFAK</td>
<td>6.16 ± 0.11</td>
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<tr>
<td>CAMWIDTH</td>
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<tr>
<td>Cortical bone, mmol/g</td>
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<td>CAMWIDTH</td>
<td>0.655 ± 0.030</td>
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Directly, our data suggest that appetite regulation is more sensitive to Zn deficiency than bone metabolism, at least in aged rats. A marginal-Zn supply at the lowest limit for normal food consumption was, apart from the subtle changes in cancellous BMDD, not associated with any negative long-term bony effects. It is clear that the lack of any major physiological effect of marginal Zn supply on bone in this study does not preclude pharmacological effects of Zn on bone metabolism. For example, several studies have shown that administration of Zn as Zn sulfate or Zn-chelating dipeptide could prevent the development of ovariectomy-induced osteopenia in rats (45–47).

In conclusion, our study strongly suggests that Zn does not play an essential role in bone metabolism in aged rats and casts doubt on the hypothesis that Zn deficiency is a risk factor for osteoporosis or other bone diseases in adult humans.

Acknowledgments
We thank Sieglinde Hirmer, Claudia Bergow, Karin Begsteiger, Michael Geritschke, and Siegfried Strohmaier for excellent technical assistance. We also thank G. Dinst, S. Thon, P. Messmer, and D. Gabriel for the sample preparations and qBEI measurements.

Literature Cited