Marginal Zinc Deficiency Exacerbates Bone Lead Accumulation and High Dietary Zinc Attenuates Lead Accumulation at the Expense of Bone Density in Growing Rats

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Environmental lead exposure is associated with reduced bone growth and quality, which may predispose to osteoporosis. Zinc supplementation may reduce lead accumulation; however, effects on bone development have not been addressed. Our objective was to investigate the effects of marginal zinc (MZ) and supplemental zinc (SZ) intakes on bone lead deposition and skeletal development in lead-exposed rats. In a factorial design, weanling Sprague-Dawley rats were assigned to MZ (8 mg/kg diet); zinc-adequate control (CT; 30 mg/kg); zinc-adequate, diet-restricted (DR; 30 mg/kg); or SZ (300 mg/kg) groups, with and without lead acetate-containing drinking water (200 mg Pb/l) for 3 weeks. Excised femurs were analyzed for bone mineral density (BMD) by dual-energy x-ray absorptiometry, morphometry, and mineral content. MZ had higher femur lead and lower femur zinc concentrations and impaired skeletal growth and mineralization than CT. DR inhibited growth but did not result in higher femur lead concentrations than CT. SZ had higher femur zinc and lower femur lead concentrations than the other treatments. DR and SZ had impaired BMD versus CT and MZ. Lead also retarded skeletal growth and impaired BMD, but an interaction between lead and MZ was only found for femoral knee width, which was lower in MZ exposed to lead. In summary, while MZ deficiency exacerbated bone lead concentration, it generally did not intensify lead toxicity. SZ was protective against bone lead but was detrimental to BMD, suggesting that the optimal level of SZ to reduce lead absorption, while supporting growth and bone development, requires further investigation.

Key Words: zinc deficiency; zinc supplementation; lead; bone; rats.

Chronic exposure to environmental lead remains a significant public health issue among low-income populations (Tong et al., 2000). While much attention has been paid to the effects of lead on neurocognitive function in children, new research on lead and bone development has raised serious concerns with respect to bone growth and quality. In fact, there has been a growing interest in the effects of childhood lead exposure on various health outcomes throughout adulthood. Chronic lead exposure has been linked with cardiac arrhythmia (Cheng et al., 1998), renal insufficiency (Lin et al., 2003), hypertension (Hu et al., 1996), and now, osteoporosis (Campbell et al., 2004). In rats, lead exposure has been associated with reduced bone strength (Ronis et al., 2001) and impaired bone mineral density (BMD) (Escribano et al., 1997; Puwals et al., 1999), but studies in adults and children have reported either no effect (Alfven et al., 2002; Laraque et al., 1990) or an increase in BMD (Campbell et al., 2004). However, a lead-induced increase in BMD may be related to accelerated bone maturation, which could interfere with peak bone mass achievement and increase the risk of osteoporosis later in life (Campbell et al., 2004).

Bone metabolism is critical to the fate of lead introduced to the body (Peraza et al., 1998). Lead accumulation in the skeleton begins during fetal bone development and continues throughout life (Hamilton and O’Flaherty, 1995). However, the developing skeletal system is much more sensitive to toxicity than that of the adult (Pounds et al., 1991). Elevated blood lead levels in children have been associated with reduced height, weight, and chest circumference (Kordas et al., 2004b; Schwartz et al., 1986) in various epidemiological studies, with the strongest associations reported between blood lead level and height (Schwartz et al., 1986).

Poor nutritional status, while not a causative agent in toxicity, can increase the risk of adverse health effects from environmental lead exposure (Mahaffey, 1981). Dietary deficiencies of calcium, iron, and zinc have been shown to enhance lead absorption in rat models (Mahaffey, 1981). However, studies examining the association between nutritional status and lead toxicity in humans have produced equivocal results, with some positive effects on blood lead levels (Hammad et al.,...
supplementation at 300 mg/kg was selected as a high, but nontoxic, dose in growing rats. All dietary treatments, except DR– and DR+ were provided ad libitum. Experimental diets were modified AIN-93G diets, containing egg white, additional biotin (2 mg/kg), and potassium phosphate (5.4 g/kg diet for the growth formulation), as previously described (LePage et al., 1999) and necessary to deliver the MZ diet. Zinc was added to each diet as ZnCO₃ according to the desired concentration for each dietary group. All diets were powdered and made in stainless steel bowls that had been previously rinsed with distilled water. Rats were housed individually in stainless steel hanging cages, and special precautions were taken to avoid zinc and lead recycling and contamination throughout the experimental period. Lead-treated animals were housed on a separate cage and low zinc groups housed above groups with higher zinc diets.

Feed intake and water intake were measured throughout the study, and diet spillage was recorded. Body weight was recorded weekly for all groups, except after day 10, when it was recorded daily in order to restrict the feed intake of the DR– and DR+ rats to match the weight of the MZ– and MZ+ treatments, respectively. The protocol for animal care procedures was approved by the University of Manitoba Protocol Management and Review Committee.

Tissue collection. All animals were euthanized by CO₂ asphyxiation and exsanguination in accordance with the guidelines of the Canadian Council on Animal Care (1993). Body weights were recorded, and trunk blood was collected. Blood samples were stored on ice until centrifuged (Beckman TJ-6R centrifuge) at 1290 × g for 15 min to obtain serum. Livers were dissected, briefly rinsed with phosphate-buffered saline to remove superficial blood, and weighed. Tissues were frozen in liquid nitrogen and stored at −80°C, along with the serum samples. Rat carcasses were stored at −20°C for high-resolution scanning and thawed prior to all in vivo scans. Right femurs were then excised, cleaned of soft tissue, and scanned ex vivo.

Bone morphometry. Femur measurements were obtained with digital calipers to the nearest 0.01 mm as previously described (Reichling and German, 2000) and included length, diaphysis thickness, and femoral head, neck, and knee joint width. All measures were reproduced in triplicate by the same examiner.

Mineral analysis. After obtaining wet and dry weights, organs and diet samples were wet digested using trace element–grade nitric acid (3 ml per tissue and diet sample), as previously described (Clegg et al., 1981). Acid digests were diluted appropriately with double-deionized water before analysis of lead and zinc (all tissues and diets), calcium and phosphorus (femurs), and copper (liver) by inductively coupled plasma atomic absorption analysis (Varian Liberty 200, Varian, Mississauga, Ontario, Canada). Bovine liver standard reference 1577b (National Institute of Standards and Technology, Gaithersburg, MD) was processed in triplicate as a quality control. Detection limits for zinc, lead, copper, calcium, and phosphorus were 0.1, 0.5, 0.1, 0.1, and 0.5 ppm, respectively.

Dual-energy x-ray absorptiometry scans. The whole body and spine of rat carcasses were analyzed for bone area (BA), bone mineral content (BMC), and BMD in situ by dual-energy x-ray absorptiometry (DXA, 4500A; Hologic Inc., Bedford, MA; small-animal software high-resolution option). Animals were placed dorsally, in an anterior-posterior position. DXA has demonstrated good precision and accuracy in measuring BMC and BMD in small animals, in situ (Lochmüller et al., 2001), as well as in isolated small-animal bones (Kastl et al., 2002). The precision error (as coefficient of variation: CV%) for triplicate scans of BA, BMC, and BMD was within an acceptable range at 4.0, 2.9, 1.5%, respectively, for the spine and 1.8, 1.5, 0.4%, respectively, for the whole body. Excised femurs were also analyzed for BA, BMC, and BMD by DXA. Femurs were put in a plastic water bath with 2 cm of water above the bone and aligned in the anterior-posterior position. The water bath was tested for interference with the scan accuracy. The precision error (as CV%) for triplicate scans of excised femur BA, BMC, and BMD was within acceptable limits at 2.0, 1.0, and 1.1%, respectively.

Biochemical assays. Osteoblast and osteoclast activities were measured by an ELISA specific for rat osteocalcin (Rat-Mid Osteocalcin, Osteometer
Biotech, Herlev Hovengrade, Denmark) and bone-related plasma degradation products of C-terminal peptides of type I collagen in rats, respectively (Ratlaps, Osteometer Biotech). Osteocalcin is considered a marker of osteoblast activity (bone formation), while C-terminal peptides of type I collagen is a marker of osteoclast activity (bone resorption). The assay was carried out according to the manufacturer’s instructions. Samples were analyzed in duplicate and agreement was > 85%.

Statistical methods. Data were analyzed for main effects of lead and zinc, as well as interactions of lead and zinc, by two-way ANOVA using SAS software version 9.1 (SAS Institute, Cary, NC). However, when no lead was detectable in the tissues of non–lead-treated animals, a one-way ANOVA was used to analyze the MZ+/CT+ groups only. Repeated measures analysis was performed for main effects and interactions on weekly body weights and growth rates. Data were checked for normality and homogeneity of variance and transformed when necessary, although non-transformed means are reported. For main effects, significant differences among treatment group means were determined with preplanned contrasts. Differences were considered significant at \( p \leq 0.05 \). All data are reported as means ± SEMs. If there was no interaction between lead and zinc, tables and figures report means of main effects for lead and zinc only. When an interaction between lead and zinc was present, data are presented as means for all eight treatment groups.

RESULTS

Body Weight and Feed Intake

The initial body weights were not different when the treatment period began (103.9 ± 2.9 to 108.5 ± 2.1 g, \( p \leq 0.05 \)). Lead treatment as a main effect resulted in reduced body weight regardless of dietary zinc intake and was apparent on day 7 (Fig. 1a). Body weight of Pb+/CT+ groups was 4% lower on day 7 and 6% lower on days 14 and 21 compared to Pb−/CT− groups. There was also a main effect of dietary zinc. MZ deficiency resulted in a 6% lower body weight than that in the CT rats on day 7, which worsened to a 10% lower weight by days 14 and 21 (Fig. 1b). DR rats weighed the same as CT rats until the final week of the study when they weighed 11% less than the CT groups but had a weight equivalent to the MZ groups. SZ treatment resulted in a 5% increase in body weight in comparison to CT rats on day 14, but this difference was no longer apparent on day 21. The interaction of lead and dietary zinc over time, for body weight, did not reach significance.

There were main effects of lead and zinc on total feed intake. MZ and DR rats had 13% and 9% lower feed intakes, respectively, than CT rats, and Pb+/CT− rats had a 9% lower intake than Pb−/CT− (Table 1).

Growth rate was not affected by lead treatment but there was a main effect of zinc. Growth rate was depressed by MZD and DR (Table 1). During week 1, growth rate was 17% lower in MZ rats than CT rats. During week 2, MZ and DR rats had 24 and 17% lower growth rates, respectively, than CT rats, but during the third week, only DR had a lower growth rate (41%) than CT.

Lead and Zinc Dosage

Total lead consumed during the 3-week study was not affected by dietary zinc intake or diet restriction when calculated as a total amount (data not shown) or a total amount/g of body weight (Table 1). Lead intake ranged from 15.3 ± 2.1 to 18.7 ± 1.3 mg Pb/kg body weight/24 h and was not significantly different among the four dietary treatment groups. Dietary zinc intake was not different between MZ− and MZ+ (0.145 ± 0.002 and 0.122 ± 0.005 mg Zn/kg body weight/24 h, respectively), DR− and DR+ (0.548 ± 0.008 and 0.499 ± 0.011 mg Zn/kg body weight/24 h, respectively), or CT− and CT+ (0.589 ± 0.023 and 0.561 ± 0.023 mg Zn/kg body weight/24 h, respectively). Zinc intake was higher (\( p < 0.05 \)) in SZ− rats than SZ+/CT+ rats (6.280 ± 0.147 and 5.829 ± 0.129 mg Zn/kg body weight/24 h, respectively). However, the percent difference between SZ− and SZ+ is relatively small at only 7%. Total water intake did not differ with respect to dietary treatment group but was 13% lower as a main effect in Pb+ than Pb− rats (Table 1). However, Pb+ rats consumed 10% more water when calculated relative to body weight (Table 1).
TABLE 1

Effects of Lead, Dietary Zinc, and Diet Restriction on Feed Intake, Growth Rate, and Lead Dose

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Lead treatment</th>
<th>Dietary group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Pb</td>
<td>+ Pb</td>
</tr>
<tr>
<td>Feed intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total g/3 weeks</td>
<td>404 ± 6</td>
<td>367 ± 8*</td>
</tr>
<tr>
<td>Growth rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1, g/day</td>
<td>8.6 ± 0.2</td>
<td>7.7 ± 0.3</td>
</tr>
<tr>
<td>Week 2, g/day</td>
<td>7.2 ± 0.3</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>Week 3, g/day</td>
<td>6.9 ± 0.3</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>Water intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water intake, ml/3 weeks</td>
<td>517 ± 13</td>
<td>449 ± 19*</td>
</tr>
<tr>
<td>Water intake, ml/g body weight/3 weeks</td>
<td>0.52 ± 0.02</td>
<td>0.58 ± 0.02*</td>
</tr>
<tr>
<td>Lead dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total g/3 weeks</td>
<td>402 ± 11</td>
<td>367 ± 8*</td>
</tr>
<tr>
<td>Water intake, ml/g body weight/3 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mg Pb/g body weight/3 weeks</td>
<td></td>
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</table>

*As there was no interaction between lead and zinc but there was a main effect of lead and a main effect of zinc, data were pooled to show means of main effects only. Pb− represents MZ−, DR−, CT−, and SZ− groups; Pb+ represents MZ+, DR+, CT+, and SZ+ groups; MZ represents MZ− and MZ+; DR represents DR− and DR+; CT represents CT− and CT+; and SZ represents SZ− and SZ+. Values are means ± SEs for n = 32 (lead effect) and n = 16 (zinc effect), as determined by two-way ANOVA, except for total lead dose. Values for total lead dose are means ± SEs for n = 8 for MZ+, DR+, CT+, and SZ+, as determined by one-way ANOVA; NS: not significant.

aStatistical differences are indicated by an asterisk (lead effect).
bStatistical differences are indicated by lowercase letters (zinc effect).

cStatistical differences are indicated by an asterisk (lead effect).

Bone Mass

Differences in BA and BMC of excised femurs were similar and contributed to the changes in BMD (Fig. 2a–2c). Lead treatment as a main effect resulted in a 7% lower BMC and a 4% lower BMD, but the change in BA only approached significance. There was also a main effect of dietary zinc with MZ treatment resulting in 6% less BA and 9% less BMC than CT, although there was no change in BMD. DR had 8% less BMC and 6% less BMD compared to CT, but no change in BA. While SZ did not result in significant changes in BA or BMC, MZ and DR groups had 5% lower femoral weights than CT, as a main effect of lead but was not affected by dietary zinc or diet restriction (Table 2). Femoral head width was 2% lower in the Pb− group compared to the Pb+ group, as a main effect of lead (Table 2). As a main effect of zinc, MZ and DR groups had 5% lower femoral weights than CT. However, MZ rats had 14–16% lower femoral weight than all other treatments, when calculated relative to body weight (Table 2).

Femur Morphometry and Mineralization

Pb+ rats had a 6% lower femoral weight than Pb− treatments, as a main effect of lead (Table 2), but not when calculated relative to body weight (Table 2). As a main effect of zinc, MZ and DR groups had 5% lower femoral weights than CT. However, MZ rats had 14–16% lower femoral weight than all other treatments, when calculated relative to body weight (Table 2).

Femoral length was 2% lower in the Pb+ rats compared to the Pb− groups, as a main effect of lead (Table 2). As a main effect of zinc, MZ and DR treatments resulted in a 2% lower femoral length than the SZ treatment, but not CT.

Femoral head width was 2% lower in the Pb+ than in the Pb− groups as a main effect of lead, but was not affected by dietary zinc or diet restriction (Table 2). Femoral neck width and diaphysis width were not significantly different among treatment groups (data not shown).

There was a significant interaction between lead and dietary zinc on femoral knee width (Table 3). The addition of lead resulted in a 4% lower knee width in MZ− than MZ+ rats but did not have an effect in DR, CT, or SZ rats.

Femur and Hepatic Mineral Concentrations

Femoral calcium and phosphorus concentrations were not affected by lead treatment but there was a main effect of zinc,
Femoral lead concentration also responded to dietary zinc intake (Fig. 4b). Femur lead concentration was 62% higher in MZ+ rats than CT+ rats, while SZ+ rats had 65% less lead than CT+ rats. Femur lead concentration was not different between CT+ and DR+ rats.

Hepatic copper concentration was 13% lower in Pb+ rats compared to the Pb− treatments, as a main effect of lead (Fig. 5). There was also a main effect of zinc, with SZ treatment resulting in 32% lower hepatic copper concentrations than CT. MZ rats had 10% lower hepatic copper concentrations than DR rats, but both groups were not different from CT.

DISCUSSION

Previous studies have independently investigated the effects of lead exposure and the effects of dietary zinc on bone in growing rats (Escribano et al., 1997; Hamilton and O’Flaherty, 1995; Hosea et al., 2004; Ovesen et al., 2001), but this is the first study to examine the interaction of lead with low and high levels of dietary zinc on bone development. This study shows that dietary zinc markedly affected lead concentration in bone, which is reflective of whole-body lead burden (Peraza et al., 1998). MZ rats had more than seven times the lead concentration of SZ animals, while CT rats had almost three times as much lead as SZ rats, confirming previous observations (Cerklewski and Forbes, 1976; El-Gazzar et al., 1978). Thus, zinc supplementation is highly protective against lead accumulation in the femur. This is an important finding, as skeletal lead can be mobilized during periods of increased calcium demand and bone remodeling, such as growth and pregnancy (Pounds et al., 1991). Once lead is mobilized to the blood, it is able to act on sensitive targets within the body such as the nervous and hematopoietic systems (Peraza et al., 1998). The fact that there was no significant difference between DR+ and CT+ rats in terms of femoral lead concentration, suggesting that the dietary restriction employed in the present study did not increase whole-body lead burden is also of note. Thus, the greater lead deposition in MZ femurs appears to be a specific effect of zinc deficiency, per se, rather than the result of a lower feed intake.

While lead exposure and MZD were both detrimental to bone development, the effects were not additive, despite higher lead accumulation in MZ femurs. Lead and MZD independently resulted in less femoral weight and BMC, whole-body BA and BMC, weight gain, and feed intake. In contrast, femoral knee width was the only skeletal outcome that was lower with lead exposure in MZ rats, but not in other treatments, supporting the hypothesis that lead toxicity would have more detrimental effects with MZD than with an adequate or supplemented zinc diet. This is a potentially important finding as the decrease in knee width may reflect abnormal growth plate function and/or trabecular bone development.

Conversely, SZ resulted in much lower bone lead concentrations, but impaired BMD. BMD is a key indicator of bone.

FIG. 2. Effects of lead, dietary zinc, and diet restriction on femur (a) BA, (b) BMC, and (c) BMD of growing rats, measured ex vivo by DXA. Values are means ± SEs for n = 26–27 (lead effect) and n = 11–15 (zinc effect), as determined by two-way ANOVA. Statistical differences among means (p ≤ 0.05) are indicated by an asterisk (lead effect) or lowercase letters (zinc effect). As there was no interaction between lead and zinc but there was a main effect of lead and a main effect of zinc, data were pooled to show means of main effects only. Pb− represents MZ−, DR−, CT−, and SZ− groups; Pb+ represents MZ+, DR+, CT+, and SZ+ groups; MZ represents MZ− and MZ+; DR represents DR− and DR+; CT represents CT− and CT+; and SZ represents SZ− and SZ+.

with 4% lower concentrations in MZ rats compared to DR, CT, and SZ rats (Table 2).

Femoral zinc concentration was reflective of dietary zinc intake (MZ < DR = CT < SZ), as a main effect of zinc (Fig. 4a). Lead treatment as a main effect lowered the femoral zinc concentration in DR+ versus DR−, CT+ versus CT−, and SZ+ versus SZ− rats, but not in MZ+ versus MZ− rats.
mass used to diagnose osteoporosis (Javaid and Cooper, 2002). As BMD decreases, the risk of osteoporotic fracture rises (Javaid and Cooper, 2002). Thus, although this data cannot necessarily be extrapolated to humans, a 6% decrease in BMD over a 3-week zinc supplementation period in growing animals is a concern.

SZ feeding showed a trend toward an increase in each morphometric parameter of skeletal growth, although these effects failed to reach a level of significance. Zinc supplementation has been shown to stimulate bone growth and strength in growing rats fed a 60–mg Zn/kg diet for 4 weeks (Ovesen et al., 2001). Notably, improved growth outcomes (body weight) and mineralization, bone quality may be compromised. Additionally, there may be indirect effects on bone quality, as pharmacological levels of zinc are thought to impede copper absorption and copper is essential for collagen synthesis, a key protein in which hydroxyapatite crystallizes and hardens during bone formation (Roughhead and Lukaski, 2003). Copper status was lower in SZ rats, as measured by hepatic copper concentrations, and may have contributed to the impaired BMD.

Based on the studies cited above, the detrimental effects of SZ on bone development in the present study are likely dose related. Therefore, there may be an optimal level of SZ to reduce lead absorption while promoting bone growth and development. Zinc supplementation is thought to reduce lead absorption at the gastrointestinal level, as zinc administration does not have the same protective effect (Cerklewski and Forbes, 1976). Excess zinc may compete with lead for binding on several intestinal proteins, such as the recently identified zinc transporter proteins ZIP4 and ZnT1. Additionally, the zinc-binding proteins metallothionein and cysteine-rich intestinal protein could function in lead detoxification through sequestration of lead within the enterocyte or transport to intestinal Paneth cells for storage. However, these hypotheses lack experimental evidence, and the exact mechanism of this interaction remains unknown.

Although MZ and DR resulted in smaller femurs, the net achievement in bone mass appeared to occur through different mechanisms. MZ resulted in lower femoral calcium and phosphorus concentrations and a lower rate of bone formation, which may have contributed to the impaired BMD.

As there was no interaction between lead and zinc but there was a main effect of lead and a main effect of zinc, data were pooled to show means of main effects only. Pb− represents MZ−, DR−, CT−, and SZ− groups; Pb+ represents MZ+, DR+, CT+, and SZ+ groups; MZ represents MZ− and MZ+; DR represents DR− and DR+; CT represents CT− and CT+; and SZ represents SZ− and SZ+. Values are means ± SEs for n = 32 (lead effect) and n = 16 (zinc effect), as determined by two-way ANOVA; NS: not significant.

Statistical differences are indicated by an asterisk (lead effect).

Statistical differences are indicated by lowercase letters (zinc effect).

### TABLE 2

<table>
<thead>
<tr>
<th>Lead treatmentb</th>
<th>Dietary groupc</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Pb</td>
<td>+ Pb</td>
<td>MZ</td>
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**Whole body**

<table>
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<th>Measurement</th>
<th>0 Pb</th>
<th>+ Pb</th>
<th>p value</th>
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<tr>
<td>BA, cm²</td>
<td>51.4 ± 0.5</td>
<td>49.1 ± 0.6*</td>
<td>≤ 0.05</td>
</tr>
<tr>
<td>BMC, g</td>
<td>5.94 ± 0.08</td>
<td>5.60 ± 0.08*</td>
<td>≤ 0.05</td>
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<tr>
<td>BMD, g/cm²</td>
<td>0.116 ± 0.001</td>
<td>0.114 ± 0.001</td>
<td>NS</td>
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**Spine**

<table>
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<th>Measurement</th>
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<th>+ Pb</th>
<th>p value</th>
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<tr>
<td>BA, cm²</td>
<td>1.64 ± 0.03</td>
<td>1.59 ± 0.02</td>
<td>NS</td>
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<tr>
<td>BMC, g</td>
<td>318.50 ± 8.52</td>
<td>298.71 ± 6.92*</td>
<td>≤ 0.05</td>
</tr>
<tr>
<td>BMD, mg/cm³</td>
<td>193.99 ± 3.60</td>
<td>187.78 ± 3.70</td>
<td>NS</td>
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**Femur**

<table>
<thead>
<tr>
<th>Measurement</th>
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<th>+ Pb</th>
<th>p value</th>
</tr>
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<tr>
<td>Dry weight, mg</td>
<td>339 ± 4</td>
<td>321 ± 4*</td>
<td>≤ 0.05</td>
</tr>
<tr>
<td>Dry weight, mg/g</td>
<td>0.0118 ± 0.0004</td>
<td>0.0111 ± 0.0004</td>
<td>NS</td>
</tr>
<tr>
<td>head width, mm</td>
<td>32.12 ± 0.12</td>
<td>31.61 ± 0.15*</td>
<td>≤ 0.05</td>
</tr>
<tr>
<td>Ca, nmol/g dry weight</td>
<td>5.29 ± 0.04</td>
<td>5.26 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>P, nmol/g dry weight</td>
<td>3.45 ± 0.02</td>
<td>3.43 ± 0.03</td>
<td>NS</td>
</tr>
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</table>

### Notes

a As there was no interaction between lead and zinc but there was a main effect of lead and a main effect of zinc, data were pooled to show means of main effects only.

b Statistical differences are indicated by lowercase letters (zinc effect).

c Statistical differences are indicated by an asterisk (lead effect).
type I collagen, suggesting that DR rats undergo less modeling for growth. Therefore, the size of the bone may not be responding as quickly in these animals to accommodate for ponderal growth. This is in agreement with the growth pattern of DR rats, as their weight gain per day dropped sharply over the final days of the study.

Interestingly, BMD was not impaired by MZ, as it was with DR, which may be related to the growth patterns experienced by these animals. MZ rats had a compromised growth rate during the first 2 weeks of the study but showed some recovery during the final week. Thus, there is likely a metabolic adaptation to the low zinc supply or decreased zinc requirements with age that may help support bone development during MZD. Conversely, the growth rate of DR rats fell during week 2 and dropped severely over the final week. Therefore, the growth inhibition seen in these animals occurred more suddenly and at the end of the study, whereas MZ rats had time to adapt to their diet. These differences suggest that DR is not an ideal control for the reduced weight gain associated with MZD, in terms of skeletal growth. However, alternative approaches are lacking in this area.

Lead treatment also resulted in retardation of skeletal growth, as indicated by lower femoral weight, length, femoral head width, and whole-body skeletal area. These results confirm previous observations in animal models (Escribano

**TABLE 3**

<table>
<thead>
<tr>
<th>Dietary group&lt;sup&gt;5&lt;/sup&gt;</th>
<th>MZ</th>
<th>DR</th>
<th>CT</th>
<th>SZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb&lt;sup&gt;-&lt;/sup&gt;</td>
<td>7.12 ± 0.04</td>
<td>7.07 ± 0.06</td>
<td>7.11 ± 0.05</td>
<td>7.17 ± 0.04</td>
</tr>
<tr>
<td>Pb&lt;sup&gt;+&lt;/sup&gt;</td>
<td>6.85 ± 0.06*</td>
<td>7.04 ± 0.04</td>
<td>7.11 ± 0.05</td>
<td>7.21 ± 0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup>As there was a significant interaction between lead and zinc for femoral knee width, data are presented as means for all eight treatment groups. Values are means ± SEs for n = 8 (p ≤ 0.05), as determined by two-way ANOVA. Statistical differences between the lead and nonlead treatments within each dietary group are indicated by an asterisk.

<sup>b</sup>Statistical differences between the lead and nonlead treatments within each dietary group are indicated by an asterisk.

FIG. 3. Effects of lead, dietary zinc, and diet restriction on serum osteocalcin (a) and serum C-terminal peptides of type I collagen (b). Values are means ± SEs for n = 32 (lead effect) and n = 16 (zinc effect), as determined by two-way ANOVA. Statistical differences among means (p ≤ 0.05) are indicated by an asterisk (lead effect) or lowercase letters (zinc effect). As there was no interaction between lead and zinc but there was a main effect of lead and a main effect of zinc, data were pooled to show means of main effects only. Pb<sup>-</sup> represents MZ<sup>-</sup>, DR<sup>-</sup>, CT<sup>-</sup>, and SZ<sup>-</sup> groups; Pb<sup>+</sup> represents MZ<sup>+</sup>, DR<sup>+</sup>, CT<sup>+</sup>, and SZ<sup>+</sup> groups; MZ represents MZ<sup>-</sup> and MZ<sup>+</sup>; DR represents DR<sup>-</sup> and DR<sup>+</sup>; CT represents CT<sup>-</sup> and CT<sup>+</sup>; and SZ represents SZ<sup>-</sup> and SZ<sup>+</sup>.

FIG. 4. Effects of lead, dietary zinc, and diet restriction on femoral zinc concentration (a) and the effects of dietary zinc and diet restriction on femoral lead concentration (b). There was a significant interaction between lead and zinc for femur zinc concentration (a) as determined by two-way ANOVA and a significant effect of zinc for femur lead concentration (b) as determined by one-way ANOVA. Values are means ± SEs for n = 8. As there was a significant interaction between lead and zinc for femoral zinc concentration, data were presented as means for all eight treatment groups. Statistical differences among treatment groups (p ≤ 0.05) are indicated by an asterisk (a) and by lowercase letters (b).
et al., 1997; Hamilton and O’Flaherty, 1995; Rossi et al., 2001), as well as humans (Campbell et al., 2004; Pounds et al., 1991). The smaller width of the femoral head in lead-exposed rats is an important finding in the present study, as the active growth plate is found in this region. This is consistent with the effect of lead on the femoral knee width of MZ rats also seen in the present study, as well as a previous rodent study, which reported growth plate thickness to be smaller in lead-exposed rats than control rats, as measured by histomorphometry (González-Riola et al., 1997). In fact, the shorter stature reported in lead-intoxicated children is thought to be indicative of growth plate dysfunction (Hicks et al., 1996). A potential mechanism of this dysfunction, the inhibition of growth plate chondrocytes, has been proposed based on in vitro studies (Hicks et al., 1996). In addition, lead is localized within areas of bone mineralization and growth (Hamilton and O’Flaherty, 1995) and incorporated into hydroxyapatite crystals during calcification, where it remains until the bone is resorbed (Hicks et al., 1996). Thus, the growth plate appears to be an important target of lead toxicity.

Lead-exposed animals in this study had a lower femoral BMD than non–lead-exposed animals, which was also mirrored by lower femoral, spinal, whole-body BMC, and whole-body BA. However, previous studies examining lead-induced effects on bone mass accumulation and development have reported equivocal findings (Bagchi and Preuss, 2005; Escribano et al., 1997; González-Riola et al., 1997). One study even reported a lead-induced reduction in bone mass, as assessed by histomorphometry, but an increase in bone mass, as determined by DXA (Escribano et al., 1997). A recent cross-sectional study in children reported that children with high lead exposure (mean, 23.6 µg/dl blood) had increased BMD compared to the low lead exposure (mean, 6.5 µg/dl blood) group in the head and the third and fourth lumbar vertebrae (Campbell et al., 2004). The increased BMD was not thought to be a false reading due to the deposition of lead in bone, and the results appeared to be clinically relevant (Campbell et al., 2004). The mechanism of a lead-induced increase in childhood BMD is not known; however, the authors felt that this result is likely transient and may lead to the attainment of a lower peak bone mass in adulthood, a predisposing factor to osteoporosis (Campbell et al., 2004). Thus, lead appears to be detrimental to skeletal development, whether it occurs through inhibition of bone formation or accelerated maturation.

In summary, MZD appears to exacerbate tissue lead concentrations, while zinc supplementation was more effective than an adequate zinc diet in preventing this build-up. However, the high zinc dose used in the present study had detrimental effects on bone density. Therefore, the optimal level of zinc supplementation to reduce lead absorption and support growth and development requires further investigation. Lead appeared to target the growth plate region of the long bone, and the effect was only detrimental with MZD, not DR, CT, or SZ treatments, in the femoral knee. However, MZD generally did not intensify other measures of lead toxicity despite exacerbating tissue lead concentrations. These results have important implications for mineral supplementation trials in the treatment and prevention of lead toxicity in children and require further investigation.

FIG. 5. Effects of lead, dietary zinc, and diet restriction on hepatic copper concentration. Values are means ± SEs for \( n = 32 \) (lead effect) and \( n = 16 \) (zinc effect), as determined by two-way ANOVA. Statistical differences among groups; Pb− represents MZ−, DR−, CT−, and SZ− groups; Pb+ represents MZ+, DR+, CT+, and SZ+ groups; MZ represents MZ− and MZ+; DR represents DR− and DR+; CT represents CT− and CT+; and SZ represents SZ− and SZ+. In summary, MZD appears to exacerbate tissue lead concentrations, while zinc supplementation was more effective than an adequate zinc diet in preventing this build-up. However, the high zinc dose used in the present study had detrimental effects on bone density. Therefore, the optimal level of zinc supplementation to reduce lead absorption and support growth and development requires further investigation. Lead appeared to target the growth plate region of the long bone, and the effect was only detrimental with MZD, not DR, CT, or SZ treatments, in the femoral knee. However, MZD generally did not intensify other measures of lead toxicity despite exacerbating tissue lead concentrations. These results have important implications for mineral supplementation trials in the treatment and prevention of lead toxicity in children and require further investigation.

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