Skeletal Effects of Developmental Lead Exposure in Rats

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To identify possible direct and indirect mechanisms underlying the effects of lead on skeletal growth, 3 studies were conducted. In the first study, 1 male and 1 female pup/litter (n = 5 litters), were exposed ad libitum to 0, 825, or 2475 ppm lead acetate in the drinking water from gestational day 4 to euthanasia on day 55. Tibial strength was tested by 3-point bending and plasma levels of vitamin D metabolites were measured. A dose-dependent decrease of the load to failure was demonstrated but only in male pups. No differences in plasma levels of vitamin D metabolites were observed. In the second study, conducted to test if hormone treatment would attenuate the lead deficits, male and female pups were exposed to 0 or 2475 ppm lead acetate and then, from 30–60 days of age, received either saline vehicle, L-dopa, testosterone (males only), dihydrotestosterone (DHT, males only), or estradiol (females only). Lead exposure significantly reduced somatic growth, longitudinal bone growth, and bone strength during the pubertal period. Sex steroid replacement did not restore skeletal parameters in lead-exposed rats. L-Dopa increased plasma insulin-like growth factor 1 (IGF1) concentrations, rates of bone growth, and bone strength measures in controls while having no effect in lead-exposed pups. The third study was conducted at 100 days of age, when endocrine parameters have been shown to be normalized, to test for effects of lead exposure on bone formation during tibial limb lengthening (distraction osteogenesis, DO). Both DO gap x-ray density and proximal new endosteal bone formation were decreased in the distraction gaps of the lead-treated animals (p < 0.01). In conclusion, lead exposure reduced somatic growth, longitudinal bone growth, and bone strength during the pubertal period, and these effects could not be reversed by a growth hormone (GH) axis stimulator or by sex-appropriate hormones. Finally, lead exposure appears to specifically inhibit osteoblastogenesis in vivo in adult animals.

Key Words: lead; distraction osteogenesis; puberty; sex steroids; skeleton; growth.

There is ample evidence that lead exposure has profound adverse health effects on young children. There is strong clinical evidence linking lead exposure during pregnancy to low birth weight and correlating blood lead levels with decreased preadolescent growth rates and reduced stature (Frisancho and Ryan, 1991; Markowitz et al., 1990; McMichael et al., 1986; Schwartz, 1992; Shukla et al., 1989, 1991).

Skeletal growth is an integral component and the primary stimulator of somatic growth. It is known that lead accumulates in the skeleton throughout development (Barry, 1975; Hamilton and O’Flaherty, 1994) and localizes in areas of bone mineralization and growth (Hamilton and O’Flaherty, 1995; Mahaffey et al., 1973; Park et al., 1933). Lead can cause bone malformations in the rat and mouse fetus (Kennedy et al., 1975). It has been shown to inhibit bone formation in dogs (Anderson and Danylchuck, 1980), delay growth plate chondrocyte maturation (Zhitnikov and Mazhuga, 1988), and inhibit mineralization in vivo during ectopic bone induction (Hamilton and O’Flaherty, 1994, 1995). However, the mechanisms underlying these effects are as yet unclear.

Lead has effects on the hormonal regulation of calcium absorption and lead toxicity is exacerbated in the presence of low dietary calcium (Mahaffey, 1981). Lead also displaces calcium in the mineral bone matrix, which may affect bone quality (Pounds and Rosen, 1986). In addition, lead has direct effects on osteoblast function including inhibition of the vitamin D3-stimulated synthesis of osteocalcin, a major noncollagen constituent of bone important for mineralization (Hicks et al., 1995; Long et al., 1990, 1992; Pounds et al., 1991). It has also been suggested that lead has direct effects on local regulation of bone cell function via interference with calcium homeostasis and calcium regulated secondary messenger systems or via disruption of cAMP signals (Pounds, 1984; Pounds et al., 1991; Rosen, 1987) However, skeletal growth and quality are also under regulation by vitamin D metabolites, pituitary growth hormone (GH), insulin-like growth factor 1 (IGF1), and sex steroids, all of which have been demonstrated to be suppressed by lead exposure (Commorato et al., 1993; Mahaffey et al., 1982; Ronis et al., 1998a,b; Rosen et al., 1980).

We have demonstrated that beginning lead exposure early in rat development (gestational day 4) results in severe endocrine disruption accompanied by delayed puberty, suppression of prepubertal growth, and suppression of the male pubertal
growth spurt (Ronis et al., 1998a,b). Pubertal delay was accompanied by a dose-dependent reduction in plasma sex steroid concentrations during puberty (Ronis et al., 1998a). In addition, the growth-suppressive effects of lead were correlated with a dose-responsive reduction in plasma IGF₁ levels prior to and during puberty and a significant increase in GH content (Ronis et al., 1998b). This is consistent with a suppression of GH release since plasma IGF₁, derived from GH-stimulated synthesis of IGF₁ in the liver generally reflects mean GH output from the pituitary. A normalization of reproductive parameters and growth rates accompanied by normalization of plasma sex steroid and IGF₁ concentrations was observed in rats postpuberty (after day 75), in spite of continued exposure to high levels of the metal ion.

To determine if lead could impair skeletal growth and repair and reduce bone quality in the rat lifetime exposure model and to investigate the underlying mechanisms, 3 studies were conducted. The first study examined the effects of lead on bone strength and vitamin D metabolism during the pubertal growth spurt. The second study examined the effects of treatment with sex steroids and a GH axis stimulator during the peripubertal period on lead-affected growth and skeletal parameters. The third study examined the effects of lead exposure on uncoupled bone formation utilizing a model of limb lengthening (distraction osteogenesis, DO) postpuberty where endocrine parameters had normalized in spite of continued lead exposure (Aronson et al., 1997a,b; Ferguson et al., 1998).

MATERIALS AND METHODS

Animals and lead administration. Time-impregnated Sprague-Dawley rats, 9 weeks of age, were purchased from Harlan Industries (Indianapolis, IN). They were housed in an AAALAC-approved facility with lights on between 0600 and 1800 h under constant temperature (22°C) and humidity (50%). Animals were given ad libitum access to a standard laboratory feed (Formulab diet 5008, PMI Feeds Inc., St. Louis, MO) and exposed to lead acetate or acetic acid ad libitum in drinking water as described previously beginning on gestational day 4 (Ronis et al., 1998a). Groups of animals (n = 5) were exposed to 0.15% w/v (825 ppm) or 0.45% w/v (2475 ppm) lead acetate or to an acetic acid solution containing the equivalent amount of acetate to the highest lead dose (control/0% lead). One milliliter of 5 N HCl was added to each liter of solution to preclude precipitation of insoluble lead salts. At birth, litters were sexed and excess pups removed from litters at random to the cells numbers down to 6 male and 6 female pups per litter. Dams had access to water containing lead acetate or acetic acid until weaning. Thereafter, the offspring were exposed to the same solutions throughout the experiment. We have previously demonstrated that the blood lead concentrations achieved in rat pups in this model were in the range of 67–192 μg/dl at 0.15% lead acetate and 120–388 μg/dl at 0.45% lead acetate (Ronis et al., 1998a). All studies were approved by the UAMS Institutional Animal Care and Use Committee.

Experimental Groups

Study 1. The first study was designed to determine if developmental exposure to lead affected bone quality during the rat pubertal growth spurt. One male and 1 female pup/litter (n = 5 litters), were exposed to 0, 825, or 2475 ppm lead acetate from gestational day 4 to euthanasia on day 55, the age at which we have previously demonstrated maximal lead-associated endocrine disruption (Ronis et al., 1998a,b). The tibiae were dissected and tested for bone quality by 3-point bending. In addition, plasma levels of vitamin D metabolites were measured in the male pups.

Study 2. The second study was conducted to test the possible role of sex steroids and the GH/IGF₁ axis in the growth suppression and skeletal effects produced by developmental lead exposure during the peripubertal period. In this study, 6 male and 6 female pups/litter (n = 5 litters), were exposed to 0 or 2475 ppm lead acetate beginning at gestational day 4. From days 30–60, the offspring received various treatments outlined as follows. The male pups received either saline vehicle via osmotic pump (alzet 2ML4), L-dopa at a dose of 7.5 mg/kg injected sc twice daily (Greif et al., 1983), testosterone at 1.5 or 3 mg/kg/day via sc osmotic minipump, or dihydrotestosterone (DHT) at 1.5 mg/kg/day via minipump. The female pups received saline via pump, L-dopa at a dose of 7.5 mg/kg sc twice daily (Greif et al., 1983), or estradiol at 15 or 45 μg/kg/day via sc osmotic pump. The doses of steroids selected have been previously utilized by ourselves and others to restore physiological concentrations or produce supraphysiological levels in castrated, hypophysectomized, and xenobiotic-treated rats (Chen et al., 1997; Mabley et al., 1992; Millard et al., 1987). Body weight gain was monitored every 3 days. Longitudinal bone growth of the femur was calculated from x-rays taken at the beginning and end of the sex steroid replacement period. Bone strength was measured at the end of the experiment by 3-point bending analysis of the tibia and plasma IGF₁ concentrations were measured by radioimmunoassay.

Study 3. The third study was conducted to test for effects of developmental lead exposure on intramembranous bone formation during DO in postpubertal rats where we have previously demonstrated that plasma sex steroid and IGF₁ concentrations have normalized (Ronis et al., 1998a,b). To accomplish this, the lead exposure model was combined with tibial limb lengthening (DO). One male pup/litter (n = 4 litters), exposed to 0 or 2475 ppm throughout development, was fitted with an external fixator on the right tibia followed by a mid-diaphyseal fracture. Distraction was initiated the next morning at 0.2 mm twice/day and continued for 14 days. After euthanasia by anesthesia followed by decapitation, the experimental and control tibiae were removed for high-resolution radiography and decalcified histology.

Strength testing. After euthanization, the legs were disarticulated at the knee and fresh frozen with the soft tissue and fixators attached. Prior to testing, the tibiae were thawed and macerated under a dissecting microscope. For 3-point bending analyses, selected tibiae were tested to failure in the mid-sagittal plane with an MTS machine (Bionix™), operated at a constant rate of 0.25 mm/sec and work to failure was recorded (Aronson et al., 2001).

Plasma levels of vitamin D metabolites and IGF₁. Trunk blood was taken at sacrifice in heparinized tubes and centrifuged for 30 min at 3000 × g. Concentrations of selected molecules in plasma were determined by radioimmunoassay (RIA) kits from Incstar, Stillwater, MN (25 hydroxyvitamin D and 1,25 Dihydroxyvitamin D) and Diagnostic Systems Laboratories, Webster, TX (IGF₁). Samples were assayed in duplicate using 200 μl of undiluted plasma or dilutions allowing values to fall within the standard curve. The samples were stored at −20°C and thawed once for assay.

Distraction osteogenesis. All rats were handled by animal care personnel 5–7 days prior to the surgeries (Aronson et al., 1997b; Lumpkin et al., 1996). Stainless steel ring fixators (scaled down from the canine model) were surgically applied to the right tibiae, which were then fractured (Aronson et al., 1997a,b; Lumpkin et al., 1996). In all age groups tested, this protocol resulted in a standardized alignment by the fixator of the normal tibial curvature and reduction of the mid-diaphyseal fractures. The rats were returned to their cages for observation during recovery from anesthesia. The first morning after surgeries, distraction was initiated (1 day latency) at 0.2 mm bid (1000 and 1600 h) and continued for a 16-day distraction period. The rats were euthanized with an overdose of Nembutal, and both operated and contralateral tibiae were surgically removed by disarticulation at the knee and ankle. In order to facilitate careful soft tissue removal, while preserving the distraction gap tissues, the fixator was first removed by cutting the transfixing needles. The specimens could then be imaged fresh, directly on the radiographic film and high-resolution radiography performed without magnification artifact. The
specimens were then marked at the proximal ends with India ink and fixed in 10% neutral buffered formalin. With the bulk of soft tissue removed, better fixation is obtained for cell detail.

Following 5 days of formalin fixation, the tibiae were decalcified in 5% formic acid. En bloc specimens were oriented longitudinally and paraffin-embedded for coronal microtome sections (6–7 microns), which were used for formic acid. En bloc specimens were oriented longitudinally and paraffin-fixation is obtained for cell detail.

**Radiographic analysis by video microscopy.** A Xerox Micro 50 closed system radiography unit (Pasadena, CA) was used at 40 kilovolts (3 mA) for 20 s with Kodak X-OMAT film to radiograph the tibiae. This setting maximized bone detail while minimizing soft tissue interference. The tibiae were placed directly on the film at a distance of 50 cm from the source. Comparison of distraction gap radiodensities was made by videomicroscopy using Media Grabber 2.0 video capture board (Raster Ops, Santa Clara, CA) and Image Analysis 1.49 (NIH, Bethesda, MD). The mineralized bone area within the distraction gap was measured by selecting and outlining regions with a radiodensity equivalent to or greater than adjacent medullary bone. This bone density threshold was controlled for potential local variations by comparison with the analogous region in the unoperated contralateral tibia. Nonmineralized tissue regions were also demarcated within the distraction gap when the radiodensity was less than the bone in the medullary canal. The measured distraction gap area was outlined from the outer corners of the 2 proximal and the 2 distal cortices forming a quasirectangular space. Three zones of bone formation are defined within this distraction gap: (1) the fibrous interzone (FIZ) located in the middle of the gap and filled with fibroblastic cells within parallel collagen bundles, (2) the primary matrix front (PMF) located on both borders of the FIZ and where cell proliferation and new osteoid deposition occurs, and (3) the zone of microcolumn formation (MCF) where the osteoblasts become embedded in parallel mineralized bone columns that are separated by new sinusoids (Aronson et al., 1997a,b). The percentage of mineralized bone area within the distraction gap was calculated by dividing the mineralized bone area by the total gap area.

**Histology.** After radiography, the tibiae from both operated and contralateral legs were decalcified in 5% formic acid. The specimens were paraffin-embedded and cut into 6–7 micron longitudinal (coronal) sections on a microtome for staining (H & E). The samples chosen for analysis were sections selected that represented a central or near-central gap location. This was accomplished by choosing slides that contained all 4 full thickness cortices with intact marrow spaces at both the proximal and distal ends. We defined intercortical new bone as that arising from the individual cortices, peristeal new bone as that outside of each cortex, and endosteal new bone as that within cortices. In several specimens, progressive sections were taken end to end from one peripheral cortex to the opposite side cortex in 50 micron increments to judge the reproducibility of our midcoronal sampling technique. For quantitation of histology, the slides were video recorded and analyzed by NIH Image Analysis 1.49 (NIH, Bethesda, MD) under low power microscopic magnification. The relative areas of tissue types (fibrous, osteoid, bone, cartilage, sinusoids, etc.) were calculated.

**Statistical analyses.** Pups were culled to the same number of male and females per litter and the litter was used as the unit of measure to take litter effects into account. Differences between group means were determined by the Student’s t-test or one-way ANOVA followed by Student-Newman-Keuls test for all pair-wise means comparisons to detect differences among groups as appropriate. An α level of 0.05 was set to determine significance.

**RESULTS**

**Study 1: Bone Strength and Vitamin D Concentration during the Pubertal Growth Spurt**

Consistent with our previous studies (Ronis et al., 1996, 1998b), rat pups exposed to lead during development displayed a significantly reduced pubertal growth spurt in the absence of reduced food intake (g consumed/kg body weight/d) (data not shown). At 55 days of age, a dose-dependent attenuation of the work to failure (newtons) was demonstrated in lead-exposed male tibiae tested for strength by 3-point bending (control vs. 0.15%; p < 0.005, control vs. 0.45%; p < 0.0025) but not in the females (Fig. 1). Since there have been suggestions that lead might alter skeletal development through inhibition of the renal mitochondrial cytochrome P450 enzyme 25-hydroxycholecalciferol, 1-hydroxylase (Mahaffey et al., 1982; Rosen et al., 1980), we examined the effects of lead on plasma concentrations of both 25-hydroxy vitamin D₃ and 1,25-dihydroxy vitamin D₃ in the lead exposed male rats using RIA. However, no effects of lead were observed on plasma concentrations of either vitamin D metabolite at any level of exposure (Table 1).

**Study 2: Sex Steroid Treatment and GH Axis Stimulation during the Peripubertal Period**

In the second study, lead-exposure significantly reduced somatic growth during 30–60 days of age, significantly re-

**TABLE 1**

Effects of Developmental Lead Exposure on Plasma Vitamin D₃ in Male Rats at Puberty

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>25-OH D₃</th>
<th>1,25-(OH₂)D₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Lead acetate</td>
<td>18.1 ± 0.6</td>
<td>64.2 ± 6.4</td>
</tr>
<tr>
<td>0.15% Lead acetate</td>
<td>21.1 ± 1.6</td>
<td>68.9 ± 10.1</td>
</tr>
<tr>
<td>0.45% Lead acetate</td>
<td>19.7 ± 1.7</td>
<td>67.8 ± 7.6</td>
</tr>
</tbody>
</table>

Note. 25-OH D₃ (25-hydroxycholecalciferol), ng/ml plasma in male rat pups 55 days of age; 1,25-(OH₂)D₃ (1,25-dihydroxycholecalciferol), pg/ml plasma in male rat pups 55 days of age.
duced plasma IGF₁ at 60 days of age (Fig. 2), significantly reduced mean longitudinal bone growth during 30–60 days of age (Fig. 3), and reduced mean bone strength at 60 days of age (Fig. 3). Chronic L-dopa treatment has been previously reported to stimulate GH production, plasma GH, and growth rates in the rat (Greif et al., 1983). In the current study, even though no significant effect was observed on the rate of body weight gain by L-dopa treatment in control rats of either sex, L-dopa-treated control pups had higher mean plasma IGF₁ concentrations (Fig. 2), higher rates of bone growth, and significantly greater bone strength (p < 0.05) (Fig. 3). In contrast, L-dopa treatment had no significant effect on bone parameters in lead-exposed pups of either sex. Testosterone treatment also had little effect on growth parameters but did increase mean bone strength (Figs. 2 and 3). Although both testosterone and DHT treatment restored plasma IGF₁ levels in lead-exposed male animals to control values, no significant effects were observed on somatic growth rate or bone growth. In lead-exposed females, estradiol treatment further suppressed somatic growth, IGF₁, and bone strength (p < 0.05) (Figs. 2 and 3).

Study 3: Effects of Lead on DO in Male Rats Postpuberty

After DO, the experimental and control tibiae were taken for high-resolution radiography and decalcified histology. For equivalent distraction gaps, the mineralized bone (radiodensitometry) was significantly lower in the 0.45% lead-exposed rats (p < 0.01, Figs. 4 and 5). Within the distraction gaps, relative tissue types were quantitated using videomicroscopy on H & E stained sections. Consistent with the radiographic findings the amount of both total (i.e., periosteal and endosteal) new bone (p < 0.05) and proximal (p < 0.05) new bone formed was decreased in the lead-exposed rats compared to the controls (Figs. 6 and 7).

DISCUSSION

From previous work it is known that developmental lead exposure to rats and humans suppresses growth processes (Commorato et al., 1993; Hammond et al., 1989; Ronis et al., 1998a,b, Schwartz et al., 1986; Shukla et al., 1989). An important element of somatic growth is skeletal growth. It is known that lead accumulates in the skeleton and has direct
Effects on osteoblast function (Pounds et al., 1991). However, skeletal growth and quality are also under regulation by a number of endocrine systems including the GH-IGF1 axis, sex steroids, and vitamin D$_3$ metabolites, all of which have also been suggested to be disrupted following developmental lead exposure. The current studies were designed to demonstrate deficits in skeletal growth and quality following developmental lead exposure in the rat lifetime model and to distinguish between possible mechanisms underlying these effects.

The data from all 3 studies demonstrate that developmental lead exposure can indeed produce significant bone toxicity in this rat model. However, consistent with studies from other laboratories such as those of Hamilton and O’Flaherty (1994, 1995), the rat appears somewhat insensitive compared to humans where a cohort study in infants has predicted significant effects on stature at blood lead concentrations of more than 10 μg/dl (Shukla et al., 1989). In the rat lifetime exposure model, blood lead values range from 67–192 μg/dl at 0.15% lead acetate in drinking water and 120–388 μg/dl at 0.45% lead acetate. At these high lead concentrations we have shown significantly reduced bone strength, somatic and skeletal growth, and impaired osteoblastogenesis. However, these studies involve relatively small numbers of animals and high doses are required to produce statistically significant data. We have previously demonstrated dose-responsive endocrine disruption during the pubertal period and suppressed growth with an extrapolated no-effect threshold level of 3–17 μg/dl in this rat model. These values fall well within the 5–15 μg/dl range of lead exposure in U.S. infants and considerably below values for children living close to lead smelters in Eastern Europe and Asia where blood lead levels of up to 120 μg/dl have been reported (Verberk et al., 1996).

The results of the first study on bone strength show that the male rat skeleton appears to be more sensitive to lead toxicity than females during the pubertal growth spurt. These data are consistent with a more severe effect of lead on somatic growth in male rats during this period of development (Ronis et al., 1998b). However, in the current study, the decrease in bone quality was not accompanied by a lead-associated decrease in plasma vitamin D$_3$ metabolite concentrations such as has been reported to occur in lead-exposed children (Mahaffey et al., 1982; Rosen et al., 1980). It is possible that the lack of effects on vitamin D homeostasis is the result of high dietary vitamin D intake. The Formulab 5008 diet contains 3 IU vitamin D/g, which is a 3-fold higher concentration than the rat RDA.

**FIG. 3.** Effects of L-dopa and sex steroid treatment on bone growth (increased tibial length between 30 and 60 days of age) (A and B) and bone strength (3-point bending) at 60 days of age (C and D) in rat pups exposed to lead during development. Data are presented as mean ± SEM for male and female pups derived from 5 litters exposed to either 0% or 0.45% lead acetate and treated with saline, L-dopa, testosterone, DHT, or estradiol as described in Materials and Methods. Means labeled “a” are not significantly different from acetate controls, means with different letters are significantly different from each other (p < 0.05) using one-way ANOVA followed by Student Newman-Keuls test.
significant reductions in bone strength independent of effects on plasma vitamin D concentrations.

In the second study, we examined the role of GH-IGF1 axis and sex steroid hormone disruption in the lead effects on somatic and skeletal growth and bone strength during the pubertal growth spurt. We have previously demonstrated a dose-dependent decrease in plasma testosterone, estradiol, and IGF1 during 30–60 days of age in rats developmentally exposed to lead in the lifetime exposure model (Ronis et al., 1998a,b). Although GH has been shown to stimulate longitudinal bone growth in a dose dependent manner, it is not clear if the effects of GH are direct or mediated via the GH-stimulated production of IGF1 by the liver or within the target tissue (Canmalis, 1983). In the current experiment, the GH axis stimulator L-dopa produced significant increases in tibial growth and bone strength in control but not lead-treated rats. This suggests that the lead effects on skeletal growth during the pubertal growth period are not secondary to GH axis disruption. In addition, although we did not include sex steroid-treated control groups in the current study and only limited conclusions can be drawn, androgens and estrogens have been shown to play a fundamental role in the maintenance of body mass (Jilka et al., 1992; Manolagas and Jilka, 1995) and androgen treatment has previously been shown to stimulate both somatic growth and longitudinal bone growth (Jansson et al., 1983). The relative lack of effects of testosterone and DHT treatment on somatic growth and skeletal parameters in the lead-exposed male pups in Experiment 2 suggests that in spite of the known stimulatory effects of androgens on skeletal growth, the peripubertal hypoandrogenization that accompanies lead-exposure in the rat lifetime model contributes little to the lead effects on skeletal growth in males. Moreover, estradiol treatment in lead-exposed female rats resulted in further significant decreases in somatic growth, plasma IGF1, and bone strength above those observed with lead alone. These data suggest that the peripubertal hypoestrogenization that accompanies developmental lead exposure also does not contribute to lead effects on skeletal growth and quality in females. The effects of estradiol treatment observed in this study are similar...
to those previously reported for estradiol treatment of rats from weanling to early adulthood by Dubuc (1976).

The results of the third study demonstrate that developmental lead exposure can specifically inhibit osteoblastogenesis in vivo in postpubertal rats where sex steroids and IGF₁ values have previously been shown to have normalized in the rat lifetime exposure model even in the face of continued metal exposure.

In summary, we have developed a rat model of developmental lead exposure that mimics the clinical effects of childhood lead poisoning including developmental delay and growth retardation. Some of the growth effects of lead in rats may be
mediated via a delay in the development of sexually dimorphic patterns of GH secretion and an overall suppression in the GH-IGF1 axis accompanied by an additional suppression of androgen production prior to and during puberty (Ronis et al., 1998a,b). However, data from the current study suggest that neither the GH axis suppression nor lead-associated hypoandrogenization appear to play a significant role in the reduced skeletal growth and strength observed in lead-exposed rats during the pubertal growth spurt. Finally, we have data for a specific inhibitory effect of lead on osteoblastogenesis in vivo and have developed a new model to study these effects involving a combination of the lead lifetime-exposure model with a rat model of distraction osteogenesis.

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