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Bisphosphonate treatment in the *oim* mouse model alters bone modeling during growth

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Abstract

Osetogenesis Imperfecta (OI) is a heritable disease, which results from an abnormal amount or structure of Type I collagen. Bisphosphonates, a class of synthetic antiresorptive drugs used in osteoporosis management, are also used to decrease fracture incidence and improve quality of life in children with OI. In this study we used the *oim* mouse to test the hypotheses that pamidronate treatment during active growth 1. produces larger, stronger, stiffer long bone diaphyses without altering bone material properties, and 2. negatively impacts longitudinal bone growth. Our results indicate that femoral cross-sectional moment of inertia in the distal metaphysis tended to increase with pamidronate treatment and that the treated bones are thicker and structurally stiffer, but shorter than their control-dose counterpar

Keywords

osteogenesis imperfecta; bisphosphonate; pamidronate; oim mouse; bone

INTRODUCTION

Osteogenesis Imperfecta (OI), a heritable disease resulting from an abnormal amount and/or structure of Type I collagen, affects 1 in 10,000 to 60,000 people in the United States (Shoenfeld 1975; Byers 1993; Primorac, Rowe et al. 2001). It is the most common hereditary bone disease (Alman and Frasca 1987), and is characterized by moderately to extremely fragile bones which may fracture due to little or no trauma (Shoenfeld 1975; Alman and Frasca 1987; Byers 1993; Paterson 1997; Primorac, Rowe et al. 2001). OI is typically classified into four syndromes (Types I–IV) based on characterizations by Sillence (Sillence, Senn et al. 1979). Although there is no cure for OI, bisphosphonates (BPs), synthetic antiresorptive therapeutics, are currently used to decrease fracture incidence and improve quality of life in children with OI (Landsmeer-Beker, Massa et al. 1997; Wang, Bank et al. 2000; Astrom and Soderhall 2002;

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Lindsay 2002; Rauch, Plotkin et al. 2003). The goal of this study was to gain a better understanding of the mechanisms by which bisphosphonate treatment benefits OI patients.

BPs are characterized by a P-C-P backbone and categorized based on the chemical nature of their side groups, which affect chelation and potency. They are not metabolized, do not accumulate in tissues other than bone, are selectively taken up at bone formation sites, and inhibit osteoclastic bone resorption (Landman, Hamdy et al. 1995; Lin 1996; Licata 2005). Consequently, the effects of BPs persist after treatment is stopped. While the mechanisms are not well understood, BPs containing nitrogen block farnesyl diphosphate synthase in the mevalonate pathway, preventing prenylation of small GTPases responsible for osteoclast polarization and formation of the ruffled border (Sato, Grasser et al. 1991; Dunford, Thompson et al. 2001; Frith and Rogers 2003; Van Beek, Cohen et al. 2003; Rogers 2004).

OI bone tissue is inherently weakened to a degree depending upon the specific genetic defect. However, the strength of any structure is a function of its geometry as well as its material and this paper considers the general hypothesis that bisphosphonates compensate for the fragility of OI bone *tissue* by improving *structural* strength. Inhibiting resorption during growth may alter bone geometry to compensate for the weakness of the OI bone material. Briefly stated, moving the structural material further from the center of the bone cross-section increases its effectiveness in resisting bending and torsional loads (Bagi, Hanson et al. 2006). As a bone such as the femur grows longitudinally, resorptive modeling removes metaphyseal periosteal bone, creating and extending the diaphysis. Simultaneously, bone is added to the periosteal surfaces of the diaphysis, and resorbed on its endosteal surfaces, enlarging its diameter and cross-sectional moment of inertia. These processes increase bone structural strength while adding marrow volume and maintaining an efficient bone mass.

Based on these concepts, we tested whether using a bisphosphonate (pamidronate) in a growing oim mouse would increase the femoral structural strength and stiffness without altering the bone tissue material properties. We further examined whether pamidronate treatment during a period of active growth in the oim mouse reduced longitudinal bone growth.

MATERIALS & METHODS

Heterozygous B6C3Fe-a/a-Colla2$^{+/oim}$ hybrid breeder mouse pairs were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were housed under constant temperature and humidity (21°C and 55%, respectively), with a 14:10 hour light:dark cycle, and fed Purina Laboratory Chow 5001 (Purina Mills, St. Louis, MO) and water *ad libitum*. Toe snips were taken at 1 week of age for identification and DNA genotyping (Pomp and Murray 1991). Based on genotype mice were assigned to wildtype (+/+), heterozygous (+/*oim*), or homozygous (*oim*/*oim*) experimental groups, and at four weeks of age randomly assigned to one of three treatment subgroups: a control-dose of phosphate buffered saline (PBS), a low dose of pamidronate dissolved in PBS (1.25 mg/kg/mo), or a high dose of pamidronate dissolved in PBS (2.5 mg/kg/mo). Weekly treatment doses were administered intraperitoneally for 8 weeks; at 12 weeks of age the mice were weighed (Table 1) and sacrificed via carbon dioxide asphyxiation. All animal procedures were approved by the U.C. Davis Institutional Animal Care and Use Committee. The desired number of animals for each subgroup (N=10) was compromised due to bones with evident fracture callus formation (primarily in the *oim*/*oim* animals) which were excluded from the study. In addition, due to the fragility of the bones, some were accidentally fractured during harvest or handling prior to biomechanical testing. To compensate breeding was continued producing more pups of all genotypes. Additional pups were put in their respective gender and genotype subgroups accounting for the unequal numbers.

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Cross-Sectional Geometry

To quantify changes in bone shape and size due to pamidronate treatment, the right femur of each mouse was harvested. Those with evident fracture callus formation were excluded from the study. The remaining fermurs were fixed in 70% ethanol for 2 days, and stained *en bloc* using basic fuchsin (Burr and Hooser 1995). The bones were embedded in methyl methacrylate using benzoyl peroxide as a catalyst, and sectioned using a diamond wire saw (Delaware Diamond Knives, Wilmington, DE). Two transverse sections 100 μm thick were cut, one at 50% of the total length (midshaft), and the other at 30% of the total length from the distal end (distal metaphysis). The mounted sections were digitized at 25X magnification using an Axiocam camera (Carl Zeiss Microscopy, Germany). ImageTool software (http://ddsdx.uthscsa.edu/dig/itdesc.html, University of Texas Health Sciences Center, San Antonio, TX) was used to measure the periosteal (p) and endosteal (e) anterior-posterior (AP) and medio-lateral (ML) diameters (D_{APp}, D_{MLp}, D_{APe}, D_{MLe}). These measurements were used to calculate the midshaft and distal metaphyseal cross-sectional moment of inertia (CSMI) assuming an elliptical cross-section (Lopez and Markel 2000):

$$CSMI=(\pi/64)[D_{AP_p} * (D_{ML_p})^3 - D_{APe} * (D_{MLe})^3]$$

Photoshop CS 8.0 (Adobe Systems, Inc., San Jose, CA) was used to remove portions of the image that did not contain bone. The images were then converted to grayscale and thresholded in ImageTool to determine the femoral cortical area (Ct.Ar).

An Olympus BH-2 microscope and a calibrated Merz grid were used to make anterior, posterior, medial, and lateral cortical thickness (Ct.Th) measurements on both the distal metaphysis and midshaft sections. One measurement was taken in each quadrant (anterior, posterior, medial, and lateral) of each section. The four quadrant measurements were averaged to produce an estimate of the mean femoral cortical thickness for the section.

Structural Properties

The left femurs were dissected free of soft tissue, wrapped in saline-soaked gauze, and stored in a −20°C freezer until use. Femurs were thawed and those with evident fracture callus formation were excluded from the study. Pamidronate's effect on longitudinal growth was assessed by measuring the overall femoral length, from the top of the femoral head to the bottom of the medial condyle, using a Mitutoyo Digimatic caliper (Mitutoyo America Corporation, Aurora, IL) accurate to ± 0.02 mm.

Structural stiffness and strength were tested in 3-point bending using a servohydraulic dynamic testing machine (Instron model 8511.20, Canton, MA) fitted with a Sensotec fatigue-rated universal load cell (Honeywell, Columbus, OH) and controlled by a LabView (National Instruments, Austin, TX) computer program set to stop at failure. The load cell range was 0–500 ± 0.1 Newtons. The test span was fixed at 4mm, and each femur was loaded at a rate of 1 mm/s with the posterior surface in compression. The 3-point bending test was conducted in displacement-control with the load point at the mid-diaphysis and the supports just before the metaphyseal flare on each end of the bone. Data were analyzed by a Matlab (The MathWorks, Inc., Natick, MA) program to calculate femoral structural stiffness, and structural strength (ultimate load),

Material Properties

The CSMI and 3-point bending test data from contralateral right and left femurs in each mouse were used to calculate the bone tissue failure stress (σ), Young's modulus (E), and yield and

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failure tissue strains using beam equations (Lopez and Markel 2000). Brittleness was defined as 100 × yield strain/failure strain (McCarthy, Raggio et al. 2002).

Statistical Analysis

The male and female data were analyzed separately. The cross-sectional geometry, length, structural and material properties were analyzed by least squares analysis of variance procedures for unequal subclass numbers, using PROC GLM of SAS (version 8.0 2001) with fixed effects of genotype, dose, and genotype-by-dose interaction. StatView was used to examine the effect of genotype and pamidronate treatment on accidental fracture of the femurs. A two factor ANOVA and Fisher Protected Least Significant Difference was used for post-hoc comparisons. Significance was defined as $p<0.05$, and a tendency toward significance was defined as $p<0.1$.

RESULTS

Cross-Sectional Geometry

At the femoral midshaft, male and female *oim*/*oim* femurs were significantly smaller than +/+ and in some cases +/*oim* femurs in terms of D_{APp}, D_{MLp}, CSMI, and Ct.Ar. In contrast, Ct.Th was not affected by genotype in males, but in females *oim*/*oim* bones were thinner than +/*oim* and +/+ bones. Pamidronate treatment, however, had no effect on midshaft D_{APp}, D_{MLp}, CSMI, Ct.Th or Ct.Ar in males or females (Tables 2 and 3).

In the femoral distal metaphysis, male and female *oim*/*oim* femurs were again smaller than +/+ and in some cases +/*oim* femurs for D_{APp}, D_{MLp}, CSMI, Ct.Th, and Ct.Ar. Pamidronate treatment had no effect on distal metaphysis D_{APp} in males or females, but increased D_{MLp} in females ($p= 0.0125$). In females (but not males) there was a tendency toward significant increases in CSMI in the distal metaphysis with pamidronate treatment ($p=0.0589$). In both males and females Ct.Th increased with pamidronate treatment in the distal metaphysis ($p =0.0537$ and $p = 0.0527$, respectively). Pamidronate increased the Ct.Ar in females (but not males) in the distal metaphysis (p0.0448).

Longitudinal Growth, Structural, and Material Properties

As expected, *oim*/*oim* femurs were shorter than their +/+ and +/*oim* counterparts in males and females (Tables 2 and 3). Despite decreases observed in the males, pamidronate treatment only significantly decreased longitudinal growth in the females ($p=0.0014$).

Male and female *oim*/*oim* femurs were less stiff, more brittle, and required less energy to fail than both +/*oim* and +/+ femurs (Table 4), and pamidronate significantly increased structural stiffness in the males ($p=0.0354$) specifically the low dose of pamidronate increased structural stiffness compared to control dose ($p=0.0160$) as did the high dose, although not significantly in the present study ($p=0.0581$). Although not significant, a similar trend was seen for females ($p = 0.1383$). The ultimate load and work-to-failure were not affected by pamidronate treatment in either males or females.

With respect to femoral *material* properties, male and female *oim*/*oim* bone tissue had higher modulus and brittleness values than +/+ and +/*oim* tissue. Pamidronate treatment did not affect any of these material properties in either gender (Table 4).

Preparation of specimens for mechanical testing was difficult due to the fragility of the bones and many fractured accidentally. While problematic, it also provided an alternative means to test the effect of pamidronate treatment on the fragility of bones having the OI phenotype. That is, the percentage of femurs that accidentally fractured serves as a measure of structural

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weakness. Using ANOVA to examine the effects of genotype (+/+, +/*oim*, and *oim*/*oim*) and pamidronate treatment (control, low, and high doses) on the fraction of specimens that accidentally fractured during preparation showed that surprisingly genotype did not affect accidental fracture incidence ($p > 0.7$). However, pamidronate treatment significantly reduced the incidence of accidental fractures: 60.6% for control-dose vs. 25.8% ($p = 0.031$) and 27.0% ($p = 0.022$) for low and high doses, respectively.

DISCUSSION

During long bone growth, metaphyseal modeling involves resorption on periosteal metaphyseal surfaces to form the slender diaphysis. We hypothesized that pamidronate treatment during growth would result in changes in bone size and shape which would compensate for the weakened bone material associated with OI. These changes in size and shape can be attributed to inhibition of metahpyseal periosteal resorption by pamidronate.

While the findings of the present study support this hypothesis, the limitations to the study must be mentioned. First and foremost despite starting with adequate and equivalent animal numbers, decreased survival of *oim*/*oim* pups and limited numbers of fracture-free bones reduced the study population. Bones with obvious fracture callus were excluded from the study to avoid biasing the data. In addition, some bones were damaged or fractured during harvesting or preparation for biomechanical testing and they were excluded from the study. The number of right femurs used for geometrical property analysis was decreased due to bad embedding, wire-saw malfunction and operator error. In addition use of contralateral femurs may have compromised accuracy, particularly in the *oim*/*oim* animals. However, gross morphological differences in the right and left femurs within any genotype group were not noted during the course of the study, and use of the right femurs for cross-sectional measurements allowed examination of the left femur fracture surfaces. The elliptical approximation used for cross-sectional area estimates represents another limitation of this study since bone cross-sections are not perfectly elliptical. Nevertheless the elliptical approximation is commonly used to calculate CSMI in bone studies and was applied uniformly to all groups in the present study. Furthermore, although pamidronate is a nitrogen-containing bisphosphonate known to inhibit osteoclastic resorption in bone, this study did not examine the effect of the therapeutic on osteoclasts specifically. Therefore, we can only infer that changes in bone size and shape are due to the antiresorptive effects of pamidronate.

In this study, males and females were analyzed separately due to gender differences in dose-response. Lin et al. found that in growing rats bone uptake of alendronate was 30–40% less in females than in males reflecting a gender-based difference in bone-uptake of the pamidronate.

As noted in the results, the cross-sectional geometry variables in females regardless of genotype or pamidronate dose were consistently smaller than males, with the exception of Ct.Th in the distal metaphysis. This may reflect sex steroid effects on bone modeling during growth. Throughout life, subperiosteal apposition is greater in human males than in females, and the periosteal bone surface responds more to testosterone while the endosteal bone surface responds more to estrogen (Garn 1970). With respect to cortical thickness, during puberty the medullary cavity width in human females decreases sharply, presumably due to endosteal bone apposition, while in males there is an increase, due to bone resorption (Garn 1970; Martin 2002). These gender differences in bone apposition and resorption may be related to future calcium demands in females during pregnancy (Syed and Khosla 2005; Sims, Brennan et al. 2006). The fact that the difference was only seen in the distal metaphysis presumably reflects the ability of this region to adapt without compromising the integrity of the overall bone structure.

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As expected genotype significantly affected almost every property measured in this study. With respect to differential bisphosphonate response based on genotype, a study by Misof et al. (Misof, Roschger et al. 2005) demonstrated that alendronate treatment did not significantly change any of the mechanical properties of *oim*/*oim* bone *tissue*, but did significantly increase the ultimate load, stiffness, and brittleness in wildtype bone.

Our results show femoral structural stiffness increased with pamidronate treatment but structural strength did not. Although, the collagen phase is generally associated with strength whereas the mineral phase is associated with stiffness., the interaction of these two phases is critical with respect to bone biomechanical properties.. It has been shown that mineral crystals in *oim*/*oim* bone are abnormal in size, shape and orientation (Fratzl, Paris et al. 1996), and that demineralized *oim*/*oim* bone is not significantly different from wildtype bone with respect to tensile measurements (Miller, Delos et al. 2007). These studies demonstrate the importance of the interaction between the mineral and organic phases in bone biomechanical properties. In the present study pamidronate did not significantly impact material properties and therefore any increase in stiffness can only be attributed to changes in bone geometry.

The lack of significant treatment-related increases in diaphyseal ultimate load in the present study may reflect the midshaft location of the test and the short treatment period relative to the duration of diaphyseal growth and development in the mice. The putative mechanism for diaphyseal cortical changes produced by bisphosphonate treatment involves inhibition of metaphyseal resorption; thus, treatment effects in the diaphysis depend on how early in the growth process treatment begins. Treating earlier and for a longer portion of the skeletal growth period would be expected to result in positive effects in the central diaphysis. This would be consistent with the observation that children with OI who receive early bisphosphonate treatment have lower fracture incidence (Lin, Chen et al. 1992; Bembi, Parma et al. 1997; Glorieux, Bishop et al. 1998; Plotkin, Rauch et al. 2000; Chevrel and Meunier 2001; Astrom and Soderhall 2002). Our data are also consistent with the results of Rauch et al. (Rauch, Cornibert et al. 2007 who observed increases in bone mass and density in the radial distal metahpysis as compared to the diaphysis in children given pamidronate. The lack of pamidronate-related increases in the midshaft cross-section may explain why the *oim*/*oim* bone was not fully restored to untreated control.

Our data indicate that bisphosphonate treatment had no significant effect on the intrinsic material properties (tissue strength, Young's modulus, and brittleness) of OI bone. However, the increased stiffness and thickness in the metaphysis, and the trend toward an increase in structural strength (especially in females) in pamidronate-treated bones relative to untreated bones, coupled with the observation of fewer bone breaks in femurs extracted from pamidronate treated mice, indicate that the changes in structural properties in pamidronate-treated bones are primarily a consequence of the altered bone structure. The increased metaphyseal periosteal diameters in the pamidronate treated bones further supports the hypothesis that pamidronate improves bone strength by inhibiting metaphyseal resorptive modeling. The observed changes in bone structure and geometry appear independent of body weight, since pamidronate had no effect on weight in either gender or any genotype group.

Inhibition of resorption is also expected to negatively effect longitudinal growth. In our study femoral length decreased with treatment, indicating a negative effect on the physes. Other studies [35,36] have shown that there is a persistence of calcified cartilage in the growth plates of *oim* mice treated with alendronate, suggesting that aminobisphosphonates may disrupt cellular processes in the growth plate during endochondral ossification. The decreases in bone length reported here were smhowever, and as Camacho et al. (Camacho, Raggio et al. 2001) noted, are outweighed by the beneficial aspects of treatment. The effect on bone length is presumably dose-dependent since no adverse effects have been documented in clinical studies

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using much smaller, therapeutic doses (Glorieux, Bishop et al. 1998;Plotkin, Rauch et al. 2000).

Our results are consistent with previous studies of the *oim* mouse model. Homozygous *oim* mice are smaller (Camacho, Hou et al. 1999) and have more frequent fractures than their heterozygous and wildtype littermates (Saban, Zussman et al. 1996). Bones from untreated homozygous *oim* mice have thinner cortices, smaller diaphyseal diameters, and reduced CSMI as compared to wildtype mice (McBride, Shapiro et al. 1998; Camacho, Hou et al. 1999). Our bone tissue property results are also in keeping with studies showing that untreated homozygous *oim* bone is more brittle than wildtype or heterozygous bone (McCarthy, Raggio et al. 2002; Misof, Roschger et al. 2005). Furthermore, our results support those of Saban et al. (Saban, Zussman et al. 1996), who demonstrated that heterozygous *oim* mice, although they appear phenotypically similar to the wildtype, have intermediate bone morphology and structural properties compared to wildtype and homozygous *oim* animals. For the majority of properties measured, heterozygous animals were not significantly different from wildtype animals (especially in males). Thus, pamidronate treatment would be expected to have less of an effect on heterozygous animals, since their bone geometry was minimally altered relative to wildtype animals. In the present study, pamidronate treatment did not affect intrinsic material properties in the heterozygous *oim* mice and due to the minimal effect on bone geometry, they were not significantly different from wildtypes even after treatment in most cases.

Based on our analysis we conclude that pamidronate treatment alters bone modeling during femoral growth in the *oim* mouse model. Presumably these changes are due to inhibition of the periosteal resorption that occurs on metaphyseal surfaces during femoral growth. Land et al. (Land, Rauch et al. 2006) similarly noted that OI children treated with pamidronate exhibited wider metaphyses in the distal femur compared with OI-matched control-dose children. Our data suggest that pamidronate treatment results in changes to the cross-sectional geometry of the bones, but not the bone tissue itself. Reducing the resorptive aspects of bone modeling during skeletal development may result in more robust bone structures that compensate for the weakened bone tissue resulting from OI genotypes.

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Table 1

Average weight (g) at 12 wks and initial number (in parentheses) of mice assigned to pamidronate treatment groups. Pamidronate treatments were control-dose (phosphate buffered saline vehicle alone), low pamidronate dose (1.25mg/kg/wk), and high pamidronate dose (2.5mg/kg/wk).

	Males			Females		
Genotype	**Control Dose**	**Low Dose**	**High Dose**	**Control Dose**	**Low Dose**	**High Dose**
+/+	31.8±0.7 (25)	31.1±0.9 (12)	31.3±0.8 (11)	24.9±0.5 (25)	24.8±0.5 (10)	25.3±0.7 (11)
+/*oim*	30.6±0.5 (19)	31.4±1.2 (11)	29.1±0.9 (9)	23.8±0.5 (20)	23.7±0.6 (12)	25.3±0.8 (11)
oim/*oim*	24.8±0.6 (8)	24.9±0.8 (10)	25.7±1.0 (10)	21.5±1.3 (8)	21.2±0.6 (12)	21.5±0.6 (10)

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Table 2

Pamidronate dose effect on overall femoral length and cross-sectional geometry properties of the femur in male mice. Data (mean ± SEM). See text for discussion of genotypic and dose significance.

MALES									
	+/+			+/*oim*			*oim*/*oim*		
	Control	**Low**	**High**	**Control**	**Low**	**High**	**Control**	**Low**	**High**
Length (mm)	15.49±0.12 (14)	15.43±0.15 (10)	15.05±0.15 (9)	15.48±0.11 (18)	15.18±0.14 (11)	15.22±0.14 (11)	14.87±0.16 (8)	14.82±0.17 (7)	14.84±0.15 (9)
MIDSHAFT									
D_{APp} (mm)	2.08±0.06 (8)	2.09±0.07 (6)	2.05±0.06 (7)	2.02±0.06 (7)	1.93±0.06 (9)	1.93±0.06 (9)	1.65±0.10 (3)	1.80±0.07 (6)	1.81±0.08 (5)
D_{MLp} (mm)	1.47±0.04 (8)	1.48±0.05 (6)	1.46±0.05 (7)	1.41±0.05 (7)	1.41±0.04 (9)	1.39±0.04 (9)	1.19±0.07 (3)	1.24±0.05 (6)	1.30±0.06 (5)
CSMI (mm^4)	0.28±0.03 (8)	0.27±0.03 (6)	0.28±0.03 (7)	0.24±0.03 (7)	0.24±0.03 (9)	0.24±0.03 (9)	0.12±0.04 (3)	0.16±0.03 (6)	0.17±0.03 (5)
Ct.Th (mm)	0.35±0.02 (8)	0.35±0.02 (6)	0.39±0.02 (7)	0.37±0.04 (7)	0.34±0.02 (9)	0.38±0.02 (9)	0.28±0.03 (3)	0.34±0.02 (6)	0.35±0.02 (5)
Ct.Ar (mm^2)	1.58±0.09 (8)	1.64±0.10 (6)	1.55±0.10 (6)	1.53±0.10 (7)	1.51±0.08 (9)	1.54±0.08 (9)	1.18±0.15 (3)	1.09±0.11 (5)	1.31±0.11(5)
DISTAL METAPHYSIS									
D_{APp} (mm)	2.26±0.08 (8)	2.30±0.08 (7)	2.32±0.08 (7)	2.15±0.08 (7)	2.28±0.07 (9)	2.18±0.07 (9)	1.75±0.16 (2)	1.87±0.09 (6)	2.12±0.10 (5)
D_{MLp} (mm)	1.43±0.05 (8)	1.44±0.05 (7)	1.50±0.05 (7)	1.28±0.05 (7)	1.41±0.05 (9)	1.41±0.05 (9)	1.23±0.10 (2)	1.26±0.06 (6)	1.34±0.06 (5)
CSMI (mm^4)	0.23±0.03 (8)	0.26±0.03 (7)	0.28±0.03 (7)	0.17±0.03 (7)	0.23±0.03 (9)	0.23±0.03 (9)	0.11±0.06 (2)	0.13±0.03 (6)	0.14±0.04 (5)
Ct.Th (mm)	0.19±0.01 (8)	0.19±0.01 (6)	0.20±0.01 (6)	0.16±0.02 (2)	0.18±0.01 (9)	0.19±0.01 (5)	0.11±0.02[a] (2)	0.15±0.01[b] (6)	0.14±0.01[a/b](5)
Ct.Ar (mm^2)	1.48±0.12 (8)	1.58±0.13 (6)	1.62±0.13 (6)	1.19±0.16 (4)	1.49±0.13 (6)	1.43±0.11 (8)	0.93±0.32 (2)	0.98±0.14 (5)	1.17±0.19 (3)

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Table 3

Pamidronate dose effect on overall femoral length and cross-sectional geometry properties of the femur in female mice. Data (mean ± SEM). See text for discussion of genotypic and dose significance.

FEMALES									
	+/+			+/*oim*			*oim*/*oim*		
	Control	**Low**	**High**	**Control**	**Low**	**High**	**Control**	**Low**	**High**
Length (mm)	15.62±0.13[a] (13)	15.24±0.15[b] (10)	15.31±0.15[a/b] (10)	15.31±0.11[a/b] (18)	15.40±0.13[a] (13)	15.02±0.13[b] (13)	15.24±0.16[a] (8)	14.58±0.16[b] (8)	14.59±0.18[b] (7)
MIDSHAFT									
D_{APp} (mm)	1.73±0.04 (7)	1.78±0.04 (8)	1.84±0.03 (9)	1.66±0.04 (8)	1.69±0.03 (11)	1.71±0.03 (12)	1.57±0.04 (8)	1.57±0.04 (6)	1.45±0.05 (4)
D_{MLp} (mm)	1.27±0.03 (7)	1.31±0.03 (8)	1.34±0.03 (9)	1.19±0.03 (8)	1.21±0.02 (11)	1.21±0.02 (12)	1.14±0.03 (8)	1.10±0.03 (6)	1.07±0.04 (4)
CSMI (mm^4)	0.15±0.01 (7)	0.16±0.01 (8)	0.18±0.01 (9)	0.11±0.01 (8)	0.12±0.01 (11)	0.13±0.01 (12)	0.09±0.01 (8)	0.08±0.01 (6)	0.07±0.02 (4)
Ct.Th (mm)	0.27±0.01 (7)	0.28±0.01 (8)	0.29±0.01 (9)	0.25±0.01 (8)	0.27±0.01 (11)	0.28±0.01 (12)	0.25±0.01 (8)	0.24±0.01 (6)	0.24±0.02 (4)
Ct.Ar (mm^2)	1.15±0.04 (7)	1.16±0.04 (8)	1.23±0.04 (9)	0.99±0.04 (8)	1.06±0.04 (11)	1.08±0.03 (12)	0.91±0.04 (8)	0.86±0.05 (6)	0.82±0.06 (4)
DISTAL METAPHYSIS									
D_{APp} (mm)	1.91±0.05 (7)	1.93±0.05 (8)	2.02±0.04 (10)	1.84±0.04 (9)	1.81±0.04 (10)	1.87±0.04 (12)	1.68±0.05 (8)	1.70±0.05 (6)	1.58±0.07 (4)
D_{MLp} (mm)	1.24±0.03[a] (7)	1.25±0.03[a] (8)	1.34±0.03[b] (10)	1.16±0.03 (9)	1.19±0.03 (10)	1.17±0.02 (12)	1.11±0.03[a] (8)	1.16±0.03[a/b] (6)	1.22±0.04[b] (4)
CSMI (mm^4)	0.15±0.01[a] (7)	0.15±0.01[a] (8)	0.18±0.01[b] (10)	0.11±0.01 (9)	0.12±0.01 (10)	0.12±0.01 (12)	0.08±0.01 (8)	0.08±0.01 (6)	0.09±0.02 (4)
Ct.Th (mm)	0.20±0.01 (7)	0.23±0.01 (8)	0.23±0.01 (10)	0.19±0.01[a] (8)	0.21±0.01[a/b] (10)	0.23±0.01[b] (12)	0.14±0.01 (8)	0.14±0.01 (6)	0.14±0.01 (4)
Ct.Ar (mm^2)	1.13±0.06[a] (7)	1.24±0.07[a/b] (5)	1.37±0.05[b]	0.97±0.05[a]	1.06±0.05[a/b] (10)	1.16±0.04[b] (12)	0.77±0.05 (8)	0.76±0.06 (6)	0.70±0.09 (3)

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Table 4

Pamidronate dose effect on structural and material properties in male and female mice. Data (mean ± SEM). See text for discussion of genotypic and dose significance.

MALES									
	+/+			+/*oim*			*oim*/*oim*		
	Control	**Low**	**High**	**Control**	**Low**	**High**	**Control**	**Low**	**High**
Stiffness (N/mm)	386+21 (17)	406±27 (10)	403±27 (10)	360±17[a] (24)	421±26[b] (11)	404+29[a/b] (9)	196±32 (7)	270±29 (9)	260±30 (8)
Ult Load (N)	47.2±2.4 (17)	47.2±3.1 (10)	46.9±3.1 (10)	39.4±2.0 (24)	44.1±2.9 (11)	41.6±3.2 (9)	18.1±3.7 (7)	19.5±3.2 (9)	21.2±3.4 (8)
Work (Nmm)	6.57±0.60 (17)	7.77±0.78 (10)	8.15±0.78 (10)	4.97±0.50 (24)	4.64±0.74 (11)	4.74±0.82 (9)	0.83±0.93 (7)	0.87±0.82 (9)	1.30±0.87 (8)
σ (MPa)	186±15 (8)	181±17 (6)	185±16 (7)	149±17 (6)	187±14 (9)	162±16 (7)	165±24 (3)	130±17 (6)	144±21 (4)
E (MPa)	1000±230 (8)	781±265 (6)	931±246 (7)	925±265 (6)	1388±217 (9)	1112±246 (7)	1878±375 (3)	2097±265 (6)	1690±325 (4)
Brittleness (%)	44.5±6.7 (8)	29.2±7.7 (6)	36.5±7.1 (7)	53.5±7.7 (6)	45.9±7.1 (7)	60.1±7.1 7(7)	94.6±18.8 (2)	52.8±10.9 (3)	70.9±13.3 (2)
FEMALES									
	+/+			+/*oim*			*oim*/*oim*		
	Control	Low	High	Control	Low	High	Control	Low	High
Stiffness (N/mm)	355±20 (16)	348±25 (10)	372±25 (10)	304±15 (27)	319±23 (12)	370±22 (13)	210±30 (7)	210±28 (7)	234±26 (9)
Ult Load (N)	38.1±1.7 (16)	37.8±2.2 (10)	39.4±2.2 (10)	28.5±1.3 (27)	32.2±2.0 (12)	33.8±1.9 (13)	15.3±2.6 (7)	16.3±2.4 (7)	17.2±2.3 (9)
Work (Nmm)	6.14±0.56 (16)	5.74±0.71 (10)	7.58±0.71 (10)	3.08±0.43 (27)	3.97±0.65 (12)	4.29±0.62 (13)	0.63±0.85 (7)	0.93±0.79 (7)	0.99±0.75 (9)
σ (MPa)	233±16 (6)	205±13 (8)	208±13 (9)	226±16 (6)	228±12 (10)	222±11 (11)	133±14 (7)	184±19 (4)	163±19 (4)
E (MPa)	1571±258 (6)	1190±223 (8)	1235±210 (9)	1473±258 (6)	1797±20 (10)	1725±190 (11)	2152±239 (7)	2411±316 (4)	2488±316 (4)
Brittleness (%)	37.5±7.8 (5)	41.1±6.2 (8)	35.8±5.8 (9)	48.6±7.1 (6)	51.2±5.5 (10)	48.4±5.3 (11)	68.3±12.3 (2)	62.3±12.3 (2)	85.4±8.7 (4)

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