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Long-Term Zoledronic Acid Treatment Increases Bone Structure and Mechanical Strength of Long Bones of Ovariectomized Adult Rats

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Abstract. Zoledronic acid (ZOL) is a highly potent heterocyclic bisphosphonate which has been shown to inhibit bone resorption in short-term experiments in young growing animals. In this investigation we have evaluated the effects of a 1-year administration to mature, ovariectomized (OVX) rats as a model for postmenopausal osteoporosis in order to elucidate (1) the temporal changes in urinary biochemical markers of bone turnover and femoral bone mineral density (BMD), (2) to measure changes of static and dynamic histomorphometric parameters and mechanical strength, and (3) to assess the preventive effects of chronic treatment with ZOL on these parameters. In urine, deoxypyridinoline increased after OVX and was significantly reduced by ZOL administration, indicative of a reduced bone collagen turnover. These changes were accompanied by alterations of tibial cancellous bone: trabecular bone volume and parameters of bone architecture were significantly augmented by ZOL and bone formation rates fell as a consequence of suppressed bone turnover, but were still measurable. No signs of "frozen bone" or osteomalacia could be detected. BMD of the whole femurs rose in sham-operated control animals (SHAM) during the entire experimental period, whereas in OVX animals, BMD plateaued after 32 weeks at a lower level. ZOL at a low dose (0.3 μg/kg/week s.c.) did not alter whole femur BMD, but at higher doses (1.5 and 7.5 μg/kg/week s.c.) BMD increased to the level of the SHAM group. A distinct pattern was noted for the distal quarter of the femur, a region rich in cancellous bone: BMD initially increased in all treatment groups except the OVX group, and at a later stage fell again at a comparable rate irrespective of treatment. Mechanical stability, as assessed by a 3-point bending test, was significantly increased by all doses of ZOL and exceeded OVX and sham-operated controls. The effects on mechanical properties were observed at a low dose which did not measurably increase femoral BMD after 1-year treatment. Multiregression analysis revealed a significant positive correlation between maximum load and BMD, and a significant negative correlation of maximum load with labeled perimeter, a marker of bone formation and turnover. No significant correlation was found with urinary deoxypyridinoline, a marker of bone resorption. The data show that mechanical testing detects improvements of functional bone quality following low dose bisphosphonate treatment which are not identified by standard DXA measurements of BMD.

Key words: Zoledronic acid — Ovariectomy — BMD — Histomorphometry — Maximum load — Long-term study

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Zoledronic acid (ZOL) is a heterocyclic nitrogen-containing bisphosphonate which potently inhibits osteoclastic bone resorption in a variety of short-term *in vitro* and *in vivo* pharmacological screening models [1]. In previous studies, administration of the compound at a daily dose of only 0.03 μg/kg s.c for 3 weeks to young ovariectomized (OVX) rats completely prevented the loss of cancellous bone from the femur and vertebrae [2]. Static and dynamic histomorphometry of cancellous bone from the tibia of intact, young, male rats treated for 10 days with ZOL (0.028–2.8 μg/kg/day s.c.) revealed a dose-dependent suppression of bone turnover and resorption, resulting in an increase in the amount of cancellous bone [3]. Compared with the reference aminobisphosphonates pamidronate and alendronate, ZOL was, respectively, at least 100 and 10 times more potent as an inhibitor of bone resorption in the rat *in vivo* [2, 3].

These results clearly demonstrated that ZOL is a potential therapeutic agent for the treatment of both benign and malignant bone disease in which excessive bone resorption is a cardinal feature. However, in order to model more closely the clinical application of postmenopausal osteoporosis, and to comply with current regulatory guidelines, more data were required on the skeletal effects of long-term ZOL treatment in OVX adult rats. Moreover, we were interested in examining in this rat model, the influence of ZOL-treatment on the interrelationship among BMD, bone histomorphometry, and bone mechanics.

Despite extensive research on bisphosphonates over the last 30 years, relatively few *in vivo* studies have been performed in the rat for longer than 3–4 months. Etidronate and risedronate, administered to OVX rats

Table 1. Biochemical markers of bone metabolism in blood and urine

Marker	Method
Blood	
Estradiol, serum	Radioimmunoassay, Diagnostic Products Corp.
Urine	
Calcium	Atomic emission spectroscopy
Inorganic phosphorus	Technicon RA-10000, standard protocol
Creatinine	Technicon RA-10000, standard protocol
Total pyridinoline crosslinks	HPLC[a] [27, 28]
Total deoxypyridinoline crosslinks	HPLC[a] [27, 28]

Radioimmunossays and autoanalyzer assays were performed according to the manufacturers recommendations
[a] Performed by Health and Safety Executive, Occupational Medicine & Hygiene Laboratory, Sheffield, UK

for 1 year, have been shown to prevent osteopenia, as measured by histomorphometric and stereological techniques [4, 5]. For alendronate, bone morphometric and mechanical data are available indicating that 6-months treatment preserves vertebral cancellous bone without detrimental effects on cortical bone [6]. Similar findings have also been reported with tiludronate [7] and ibandronate [8]. However, there is still a paucity of data on the biomechanical effects of long-term treatment with bisphosphonates on rat long bones, and few of these studies have investigated the association among bone mass, morphometry, and biomechanics.

We report the results of a comprehensive study on the effects of 1-year ZOL treatment on bone mass, architecture, and mechanical properties in adult rats with osteopenia induced by estrogen deficiency. This investigation demonstrates not only that ZOL preserves bone mass, architecture, and strength, but also provides extensive data on the temporal changes in these parameters in untreated control animals following ovariectomy.

Materials and Methods

Animals

A total of 144 3-month old female Sprague Dawley rats were housed under standard laboratory conditions (temperature 21 ± 2°C, humidity 55 ± 10%, lighting cycle 12 h light/12 h dark). All animals had free access to tap water and were fed a diet containing 0.71% calcium, 0.5% phosphorus, and 600 IU/kg vitamin D_3.

One group of 20 rats, chosen at random, was sacrificed for baseline histomorphometric evaluation. In the remaining rats, whole body BMD was determined by dual-energy X-ray absorptiometry (DXA) and those animals furthest from the mean were discarded, leaving a pool of 100. These animals were then divided into 5 groups of 20 so that the group mean whole body BMD values were equal.

Ovariectomy on 4 groups (OVX), or a sham operation on the fifth group (SHAM), was performed under anesthesia (midazolam/fentanyl/fluanisone mixture i.p.) when the rats were 4 months old. Immediately after surgery, while the animals were still anesthetized, treatment of the 4 OVX groups was initiated with ZOL (dissolved in sterile saline) at doses of 0, 0.3, 1.5, and 7.5 μg/kg, injected s.c. (1 ml/kg) once a week for 52 weeks. The SHAM group received saline injections at an identical dosing regimen. Food consumption was recorded weekly for the SHAM group and this amount was then fed to the OVX rats over the following week. Body weight was recorded at weekly intervals throughout the study, and the wet weight of the uterus was recorded at necropsy. Both tibiae and both femurs were removed and freed of soft tissue. Tibial length was measured with callipers. The tibiae destined for histomorphometric analysis were stored in 70% ethanol. The femurs destined for mechanical testing were wrapped in gauze soaked in Ringer's solution and then frozen.

DXA Studies. Prior to surgery, the rats were subjected to DXA scans of the whole body and femur using a Lunar DPX-L and associated small animal software. BMC and BMD were then calculated. Analysis of the distal quarter of the femur was done using the femur scans. A region of interest box was placed around the distal femur using the Lunar Small Animal Software. Rats received further DXA scans during weeks 4, 17, 32, and 52 after commencement of dosing. The reproducibility and precision of total body BMD determinations were 2.6% and 0.8%, respectively. Accuracy was determined by comparison of BMC with ash weights. The coefficients of determination r^2 were 0.992 ($P < 0.001$) for total body and 0.793 ($P < 0.001$) for femur.

Histology. For the measurement of longitudinal bone growth and bone formation rate, bone-seeking fluorescent markers were injected into all rats. One day before starting treatment, the animals received calcein (20 mg/kg i.p.) to label preexisting bone. Towards the end of the treatment period, another injection of calcein (20 mg/kg i.p.) was given 17 days before necropsy followed by 2 injections of demeclocycline (20 mg/kg i.p.) at 14 and 4 days before necropsy. Histomorphometry was performed on 10 animals from each group using the procedures described previously in a study on the effects of short-term ZOL treatment in young, intact rats [3]. In brief, serial sections of 8 μm thickness were cut on a Polycut S microtome (Reichert & Jung, Germany) from the methylmethacrylate-embedded material. Sections from undecalcified bone were stained by the method of Goldner and von Kossa [9] and decalcified bone sections were stained with the Ponceau acid-Fuchsin [9]. Standard static (Tb.Ar, Tb.Wi, Tb.N, Tb.Sp, N.Nd, Os.Pm) and dynamic (BFR, MAR) histomorphometric parameters were determined for cancellous bone in the proximal tibia at least 1 mm distal from the growth plate, as detailed by Pataki et al. [3]. The evaluations were performed semiautomatically with a Leitz DMRBE microscope equipped with a video camera and a Quantimet 500 image analysis system. Fluorescence measurements were performed with a Leica Microvid system connected to a Orthoplan fluorescence microscope (Leitz, Switzerland).

Biochemical Measurements

For the determination of biochemical markers of bone metabolism, blood (serum) and overnight urine samples were collected prior to ovariectomy and during weeks 16 and 51 of

Table 2. Longitudinal growth of the tibia

Treatment	Tibia length (mm)	Longitudinal growth, demeclocycline labeling (μm/day)	Longitudinal extension of cancellous bone (mm)
SHAM	41.8 ± 0.2 (19)	4.6 ± 0.3 (5)	4.7 ± 0.2[a] (10)
OVX	42.6 ± 0.3 (19)	nm	2.5 ± 0.2 (10)
0.3 μg/kg ZOL	42.1 ± 0.3 (16)	4.1 ± 0.4 (9)	5.0 ± 0.2[a] (10)
1.5 μg/kg ZOL	41.8 ± 0.2 (17)	3.9 ± 0.4 (6)	5.3 ± 0.2[a] (10)
7.5 μg/kg ZOL	41.6 ± 0.2[a] (20)	2.9 ± 0.4[b] (5)	5.1 ± 0.3[a] (10)

Means ± SEM; (n): animals per group; statistical significance: [a] $P < 0.05$, compared to OVX rats; [b] $P < 0.01$, compared to sham-operated rats, nm: not measurable (no evaluable calcein label left).

ZOL treatment. For urine collection, rats were kept in urinalysis cages from approximately 4 p.m. to 8 a.m. the next day. Plain collection tubes were used and animals had access to water but not food. Samples of blood were withdrawn from the tail vein into plain tubes and serum was collected. The parameters measured and the assays used are listed in Table 1.

Biomechanical Testing

Immediately prior to testing, the femurs were removed from freezer storage and equilibrated overnight at room temperature in Ringer's solution. The femurs were tested by placing each across the support struts of a three-point bend test rig with the struts placed 17.5 mm apart. The test rig was immersed in Ringer's solution at room temperature on the lower crosshead of an Instron 1185 Universal Testing machine. The femurs were then tested to structural failure by applying a load at the midpoint of the support span at a constant crosshead speed of 2 mm min^{-1}. From the load deformation curve thus generated, maximum load to failure, deformation to failure, structural stiffness, and energy absorption capacity could be calculated.

Statistics

Data from the DXA scans and mechanical testing were analyzed using parametric methods. Initially the data were subjected to analysis of variance, and the variance homogeneity was checked with Bartlett's test. Dunnett's test was used to compare the saline-treated SHAM group and ZOL-treated groups against the saline-treated OVX control.

For the histomorphometric data, nonparametric tests were used since the data were collected from groups of only 10 animals and found not to have a normal distribution. The Kruskal-Wallis test was used for an overall comparison of groups, followed by the Mann-Whitney test with the Bonferroni correction for comparison of various pairs of groups. Statistical tests and multiple regression analysis were performed with the software packages Instat and Prism 3 from GraphPad Inc., San Diego, USA.

Compound

Zoledronic acid (ZOL) (1-hydroxy-2-imidazol-1-yl-phosphonoethyl) phosphonic acid was supplied by Novartis Pharma AG as the anhydrous free acid (molecular weight 272.1).

Results

Despite their receiving a similar amount of food as the SHAM group, all the OVX groups gained an average of 9% more body weight over the first 5 weeks after surgery and were still 5% heavier at the end of the study (not significant, data not shown). ZOL treatment was well tolerated; it had no effect on body weight and there were no clinical symptoms of intolerability. At necropsy, the uterine weight in all the OVX animals was reduced by more than 80% compared with that of the SHAM controls, indicating successful ovariectomy. In the serum samples collected at week 16 post-OVX, mean estrogen levels in the OVX groups were significantly reduced compared with the SHAM control group (data not shown). Long bones such as the tibiae grew slightly, but insignificantly longer in OVX rats than in SHAM rats (Table 2). ZOL treatment had no effect on tibial length except at the highest dose of 7.5 μg/kg which reduced tibial length back to that of the SHAM controls. At the histological level, no effects were seen on the growth plate thickness (data not shown), however, a dose-dependent and significant reduction in the longitudinal growth was observed between the proximal growth plate and the demeclocycline label in adjacent cancellous bone. The data show that during the experimental period the tibiae grew at the proximal end at an average rate of 4.6 μm per day in sham-operated rats, corresponding to an increase in length of 1.68 mm during the duration of the experiment (Table 2). Treatment with the high dose of ZOL reduced the longitudinal growth by about 37% to 2.9 μm per day compared with the SHAM controls. The determination of growth rates from the demeclocycline labeling was not possible in untreated OVX animals because of the irregularity and scarcity of label as a result of high bone turnover and osteopenia.

In urine, ratios of deoxypyridinoline/creatinine remained significantly increased in OVX rats after 51 weeks, whereas ZOL treatment produced a statistically significant, dose-dependent reduction (Fig. 1). Urinary calcium concentrations of OVX rats were significantly reduced to 179 ± 21 μg/ml compared with 288 ± 23 μg/ml of sham-operated controls (means ± SEM, $P < 0.01$). Treatment with 0.3, 1.5, and 7.5 μg/kg ZOL further diminished calcium to 119 ± 15, 77 ± 8, and

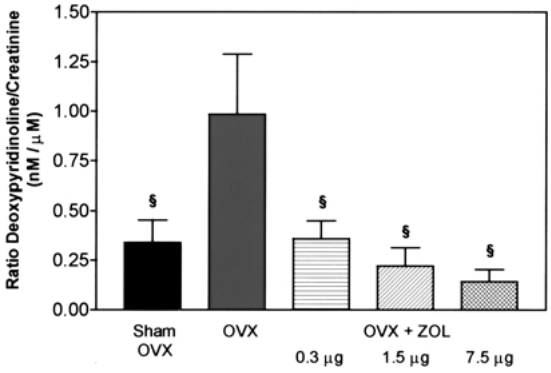

Fig. 1. Effects of OVX and ZOL administration on urinary excretion of deoxypyridinoline. Urine was analyzed 51 weeks after OVX. Bars indicate means ± SEM, OVX ovariectomized rats, OVX + ZOL ovariectomized rats treated weekly with ZOL. Statistics: §) $P < 0.05$ compared to OVX rats.

135 ± 19 µg/ml, respectively. However, only the effect of the intermediate dose was significant ($P < 0.01$, compared with OVX control rats). Phosphorus urine concentrations fell from 1.85 ± 0.16 µM in sham-operated rats to 1.15 ± 0.10 µM in OVX rats ($P < 0.01$). Treatment with ZOL for 51 weeks did not further change phosphorus levels in OVX rats (data not shown).

Taken together, these changes in urinary markers indicate an increased turnover of bone matrix following OVX which was attenuated by ZOL administration. A histomorphometric analysis of the cancellous tibial bone showed a significant increase of the osteoid perimeter, labeled perimeter, and bone formation rate 52 weeks after OVX, which was completely reversed by the administration of ZOL (Fig 2a,b,d). Concomitantly, the mineral apposition rates were slightly increased following OVX, and treatment with ZOL lowered them significantly (Fig. 2c). The effects of OVX and ZOL treatment on bone turnover were also reflected at the level of static morphometric bone parameters such as trabecular number, trabecular bone area, and node numbers (Fig. 2e,g,h). These parameters changed because of the OVX-induced loss of bone structure which was also accompanied by a characteristic diminution of the longitudinal cancellous bone extension from the growth plate towards the marrow cavity (Table 2, Fig. 3c). ZOL treatment dose dependently and significantly inhibited these changes. Interestingly, trabecular width tended to increase in OVX rats and ZOL treatment significantly reduced this effect (Fig. 2f). Photomicrographs (Fig. 3) exemplify the effects of OVX and of bisphosphonate treatment. Trabecular bone mostly disappeared after OVX whereas after treatment with ZOL the trabeculae persisted and became more densely structured.

Bone mineral density measurements at the level of the whole body or femur revealed a significantly lower gain of BMD after OVX compared with sham-operated controls (Fig. 4a,b). ZOL treatment increased the gain of whole body BMD significantly at all time points measured (Fig. 4a). The total femoral BMD increased in parallel in SHAM and OVX rats during the first 17 weeks, thereafter the BMD remained constant except in SHAM controls which continued to show a steady increase over the complete course of the experiment (Fig. 4b). The distal quarter of the femur exhibited a different pattern: in SHAM controls and ZOL-treated rats, BMD in this region increased during the first 17–32 weeks, thereafter a steady decline in BMD was noted in all groups, irrespective of treatment (Fig. 4c). Femora from animals treated with the intermediate and high dose of ZOL accumulated the highest BMD and maintained these high levels in relation to OVX or SHAM controls during the later experimental period. The lowest dose of ZOL led to a transient, initial increase of BMD over OVX control values which then returned to OVX control levels after more than 17 weeks of administration (Fig. 4c).

The mechanical impact of treatment was investigated at the end of the experiment. Femurs were subjected to a 3-point bending test (Fig. 5). A significantly diminished resistance to maximum loads and a reduced ability to absorb energy were noted in OVX rats and stiffness and deformation were insignificantly lowered. ZOL-treated OVX rats showed a significantly improved mechanical performance for all the tested properties except deformation. The intermediate dose of ZOL exerted the strongest effects.

Discussion

Ovariectomy changes the metabolic activity and longitudinal growth in the long bones of rats [10]. Although the rats were already 3 months old at the start of the study, the proximal end of their tibiae had grown by an average of 1.7 mm by the end of the study (Table 2). The proximal tibial growth plate can persist for over 3 years in rats [11] thus enabling this species to grow almost throughout life. In this experiment, growth plates were still present at 15 months of age (end of experiment), but calcified areas connecting epi- and metaphyseal trabeculae were evident in von Kossa-stained sections (Fig. 3). This shows that the animals had ceased longitudinal growth in the course of the experiment. OVX slightly, but insignificantly increased total tibial length by 0.8 mm due to accelerated endochondral growth [10] during the initial experimental phase. ZOL treatment dose-dependently reduced longitudinal growth of the proximal tibia compared with control OVX rats, and the highest dose significantly diminished the total length of the tibia back to the level of the SHAM control. However, these inhibitory effects of ZOL were minor and no significance was reached compared with the SHAM controls. Bis-

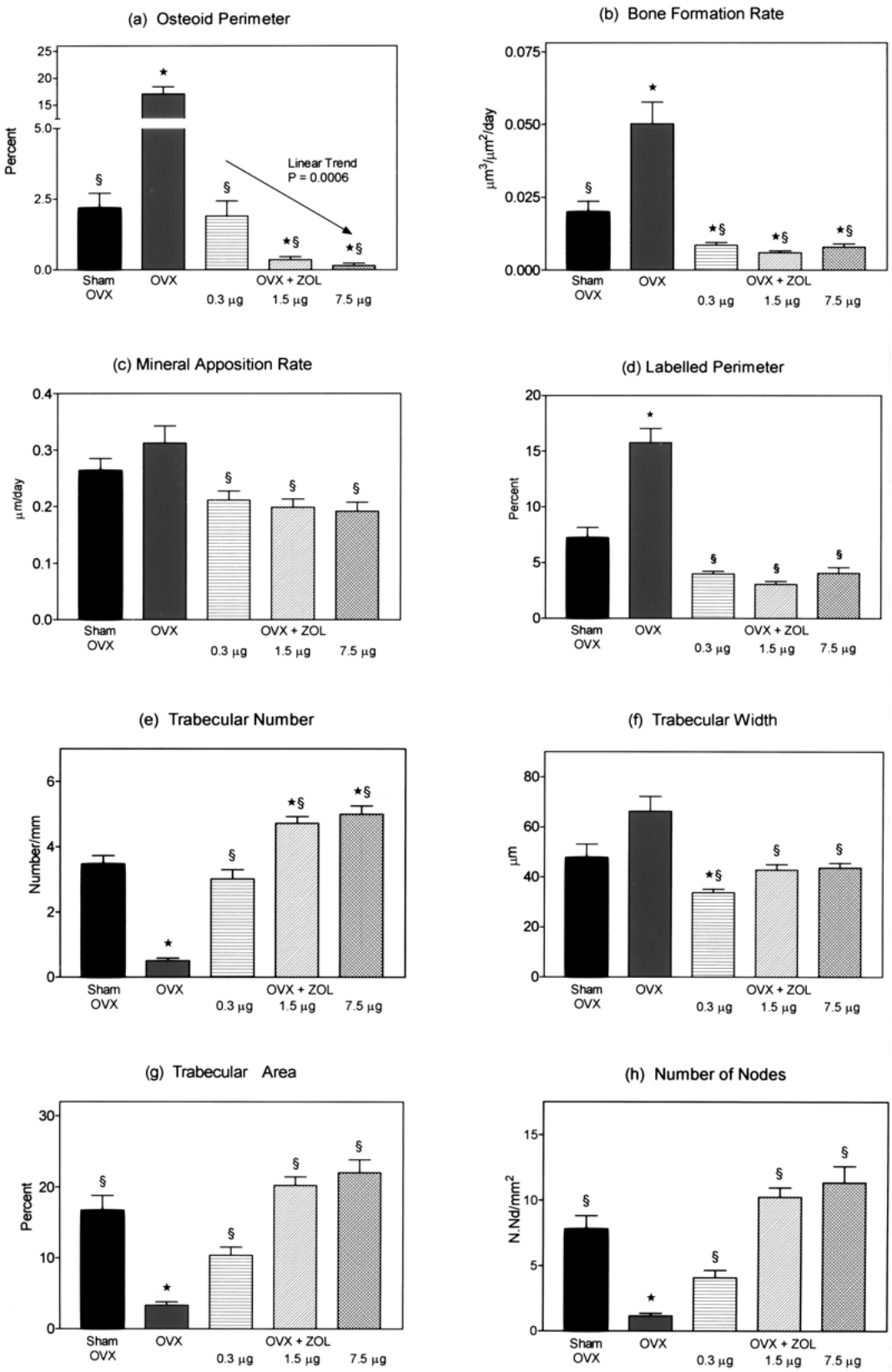

Fig. 2. Histomorphometric indices in the proximal tibia at week 52. Bars indicate means ± SEM, OVX ovariectomized rats, OVX + ZOL ovariectomized rats treated weekly with ZOL. Statistics: *) $P < 0.05$ compared to sham-operated rats, §) $P < 0.05$ compared to OVX rats.

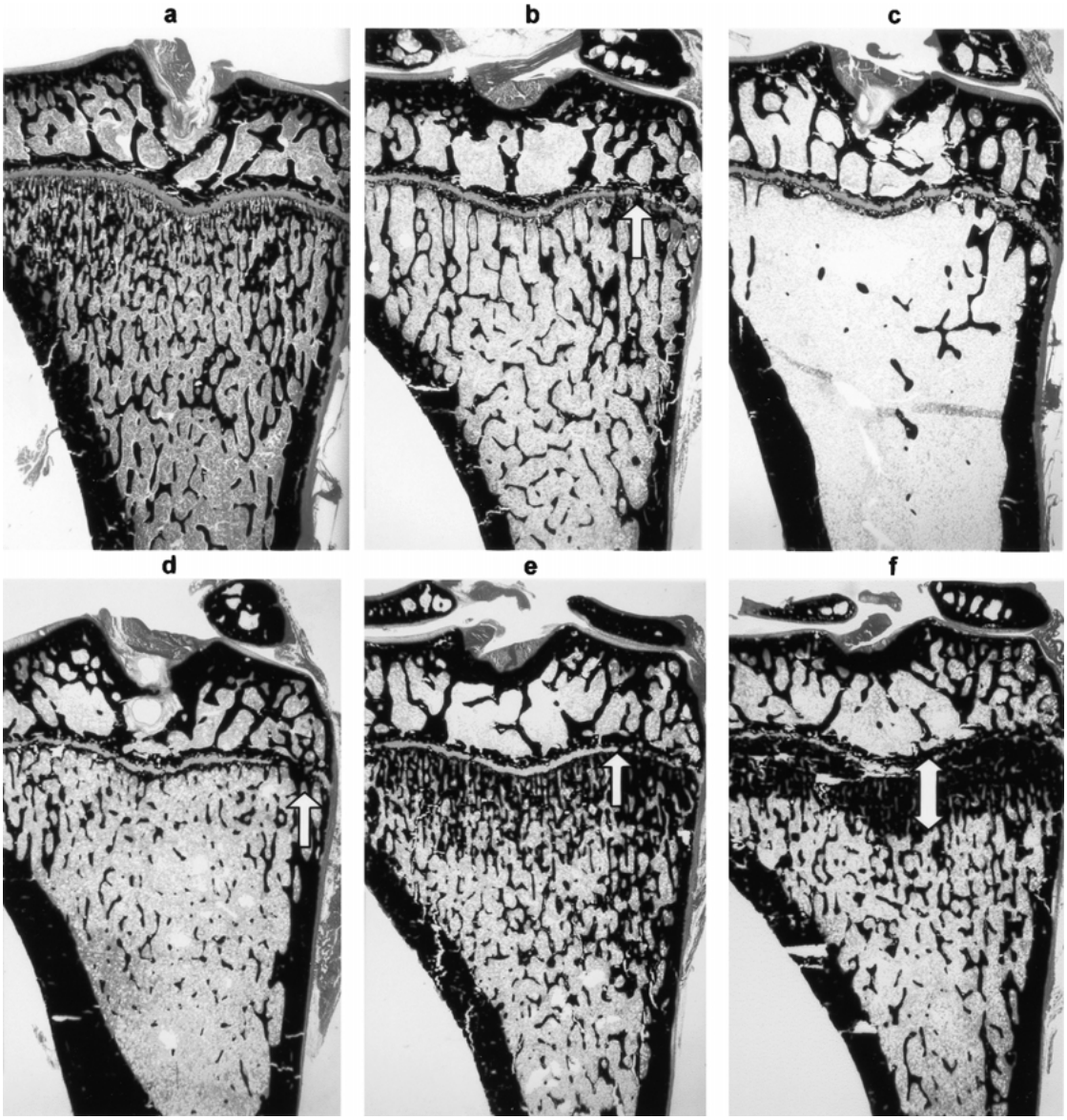

Fig. 3. Histology of the proximal tibiae at week 52. Undecalcified 8 μm sections (von Kossa) show mineralized bone tissue (black), **a**) baseline before treatment started; **b**) sham-operated; **c**) OVX; **d**) 0.3 μg/kg ZOL; **e**) 1.5 μg/kg ZOL; **f**) 7.5 μg/kg ZOL. Note in panel c) nearly complete loss of trabeculae. Calcified areas in growth plates are marked with white arrows in panels b), d) and e). Panels d)–f) show increasingly denser trabeculae under ZOL treatment. A double arrow in panel f) indicates dense spongious bone formed during longitudinal growth of the initial phase of the experiment. (Subscribers can view a color version of this figure online at: http://www.link.springer-ny.com/link/service/journals/00223/contents/02/2015/index.html.).

phosphonates at high doses are known to reduce bone length in fast growing young rats [3, 12, 13] by interfering with the modeling process.

Estrogen depletion accelerates turnover in rat long bones [10, 14] and enhances urinary excretion of collagen degradation products within weeks after surgery [15]. Here we show that even 1 year after OVX, urinary deoxypyridinoline remained markedly increased (Fig. 1) whereas other parameters of collagen turnover such as pyridinoline were not significantly altered (data not shown). Administration of ZOL completely reversed the increase in deoxypyridinoline to control levels or even below demonstrating that ZOL suppressed the high turnover of bone collagen returning it to a balanced state. Urinary calcium and phosphorus levels were significantly lower in the OVX rats. After an initial rise, calcium levels fell in OVX rats [16] and this effect has been attributed to a lack of estrogen action on the proximal tubules in rats [17]. In parallel, renal phosphate excretion is also reduced in OVX rats [18] and can be reversed by estrogen repletion [19]. Estradiol decreases the Na-P cotransport in the kidney of OVX rats [20] which is most probably the main cause for the drop in urinary phosphorus. Treatment with ZOL inconsis-

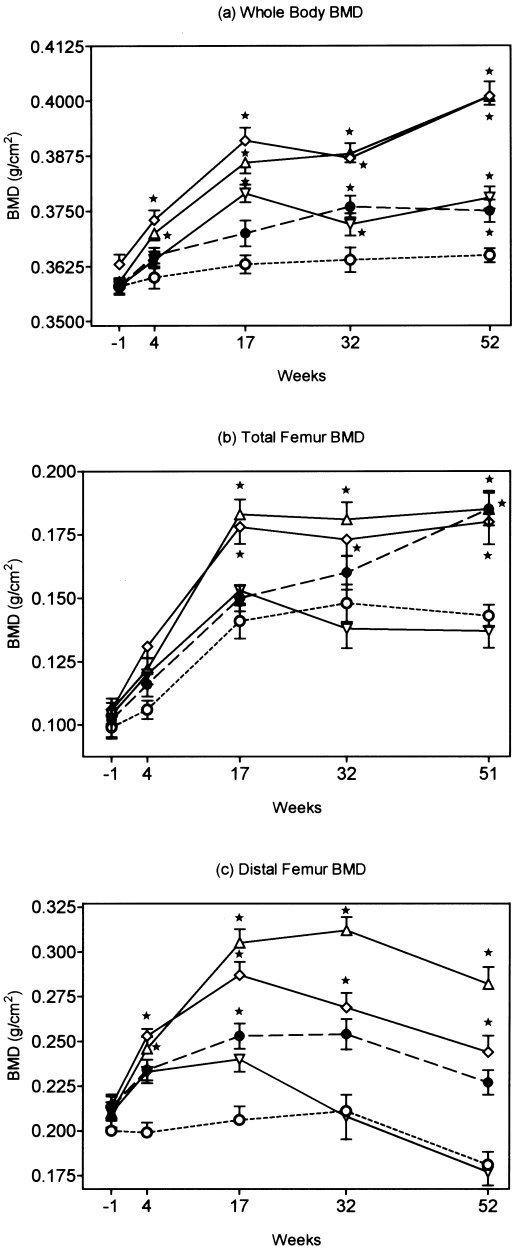

Fig. 4. Time course of BMD changes. Control groups: sham-operated, black circles; OVX, open circles. OVX + ZOL-treated: 0.3 μg/kg, inverted triangles; 1.5 μg/kg, diamonds; 7.5 μg/kg, triangles. Means ± SEM, 16–20 rats per group. Statistical significance: *) $P < 0.05$ compared to OVX rats.

tently reduced calcium concentrations in the urine and had no effect on phosphorus levels.

De novo synthesis of organic matrix was enhanced after OVX as indicated by significantly higher bone formation rates as well as by increased osteoid and labeled perimeters (Fig. 2a,b,d). These dynamic parameters point to an augmented osteoblast recruitment [21]. ZOL reduced these dynamic processes by lowering bone formation rates (Fig. 2b). However, MAR did not fall significantly below the level of SHAM controls indicating that the elevated matrix formation by osteoblasts was only reduced to normal levels, therefore the lower BFR after ZOL treatment was caused by a lower number of activated osteoblasts (Fig. 2d). It is evident from Figures 2b,c,d that bone formation was continuing at all doses tested, and no signs of reduced mineralization could be detected, in contrast to what has been reported for some other bisphosphonates [22]. The accumulation of a dense layer of spongious bone beneath the growth plate, as shown in Figure 3f, reflects the well-known pharmacodynamic effect of bisphosphonate treatment on longitudinal growth [22] during the initial phase of this experiment. The net result of these changes was an overall gain of cancellous bone mass (Tb.Ar, Tb.N)(Fig. 2e,g) and an increase of connectivity (N.Nd)(Fig. 2h).

Changes in the femoral BMD showed a different time course depending on the site measured. BMD in the whole femur steadily increased, but at the distal metaphyseal end, a site rich in cancellous bone, a biphasic pattern was observed which, with the exception of bones from untreated OVX rats, was seen in all treatment groups (Fig. 4c). ZOL administration dose-dependently increased BMD for a period of 17–32 weeks, thereafter BMD declined in all groups in a parallel manner. This was in contrast to the whole femur BMD which did not decrease during the later experimental phase. An explanation is that the compact femoral bone matured and increased its mineral density nearly normally in OVX animals (Fig. 4b), as has been shown by others for the femoral neck area where it took up to 1 year before significant changes became evident by histological methods [23, 24]. BMD in the predominantly cancellous bone of the distal femur of OVX rats increased for a limited time only (up to weeks 17–32), thereafter it decreased at the same rate, as in sham-operated controls, irrespective of continuing bisphosphonate treatment (Fig. 4c). This could possibly be the result of a readjusted biomechanical set point mechanism as originally proposed by Frost [25, 26]. After an initial phase of BMD gain, when remodeling spaces are filled, activation frequency becomes extremely small such that residual osteoclastic resorption is sufficient to remove mechanically "superfluous" cancellous bone.

Mechanical competence, as assessed by a 3-point bending test in the femur, decreased after OVX and led to a reduction in maximal load bearing, stiffness, and absorbed energy. ZOL treatment significantly prevented these reductions in a biphasic manner (Fig. 5). The contributions of BMD, labeled perimeter (L.Pm = sL.Pm/2 + dL.Pm in % of Tb.Pm, as an indicator of ongoing osteoblastic activity and bone turnover), and deoxypyridinoline (as indicator of osteoclastic activity) to maximum load were analyzed by multiple regression analysis (Fig. 6). L.Pm and BMD significantly contributed to the maximum load ($P < 0.0001$), but deoxypyridinoline made no significant contribution ($P <$

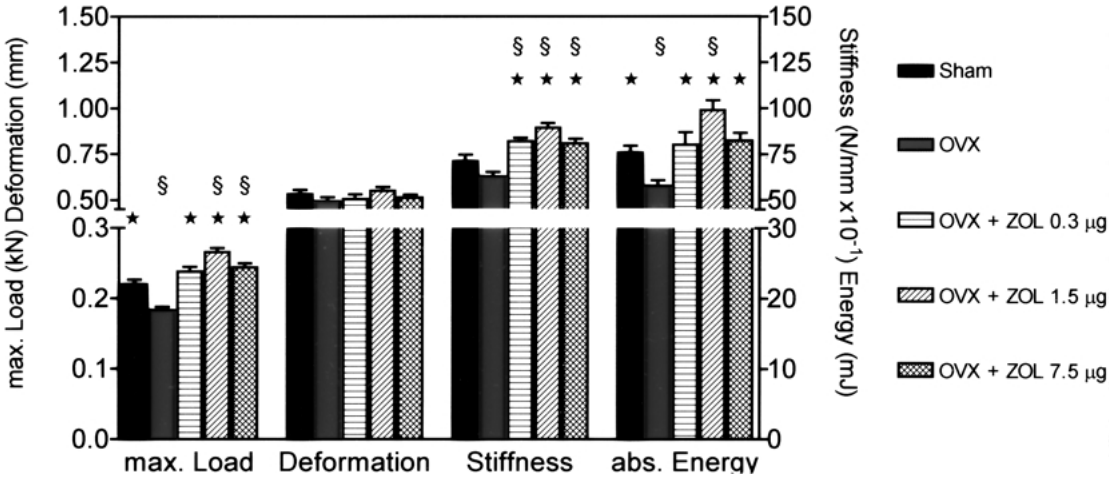

Fig. 5. Femur 3-point bending test at week 52. Bars: means ± SEM, 16–20 rats per group. Statistics: *) $P < 0.01$ compared to OVX group; §) $P < 0.05$ compared to sham-operated control group.

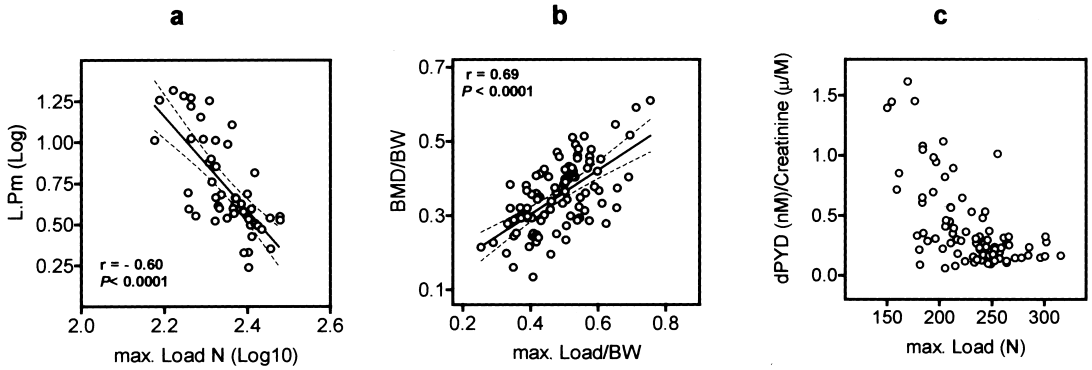

Fig. 6. Correlations with maximum load. Correlation plots of maximum load in a 3 point bending test of the femur with: **a**) labeled perimeter (L.Pm %) as a measure of osteoblastic activity; **b**) bone mineral density (BMD normalized per body weight); **c**) urinary deoxy-pyridinoline/creatinine, as a measure of osteoclastic bone collagen breakdown. Multiple regression of maximum load with variables L.Pm and BMD yields $r^2 = 66.0\%$, $P < 0.0001$. L.Pm and BMD contribute independently and significantly to the model ($P < 0.0001$). Correlation coefficients are shown as inserts in (a) and (b), L.Pm and BMD correlate with each other with $r = -0.273$.The contribution of dPYD to the regression model was not significant, $P < 0.29$ (data excluded from calculation).

0.29) and was therefore omitted from the regression model. The overall fit (r^2) to maximum load, with L.Pm and BMD as variables, was 66% ($P < 0.0001$). The data support the importance of maintaining BMD and reducing bone turnover in the femoral shaft after estrogen depletion. Labeled perimeter was determined in the cancellous bone compartment, not the cortical one, and therefore it served as a rough estimate of cortical osteoblast activity. Nevertheless, the strong negative correlation of an osteoblastic marker with maximum load underlines the importance of reducing turnover as a contributing factor for mechanical stability. To our knowledge, this is the first report in which very low doses of bisphosphonate were administered such that whole femur BMD did not significantly increase, compared with vehicle-treated OVX controls, and yet, mechanical properties were significantly improved. Precision and reproducibility of our BMD measurements were in the range of 3.5%, therefore either smaller changes in BMD were sufficient to significantly increase femoral strength or changes in cortical architecture occurred which evaded our measurements. The results indicate that an extended analysis of the long-term effects of low dose bisphosphonates on cortical bone is warranted using biomechanical rather than BMD parameters.

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