



Prostaglandin E₂ Adds Bone to a Cancellous Bone Site with a Closed Growth Plate and Low Bone Turnover in Ovariectomized Rats

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Abstract

The objects of this study were to determine the responses of a cancellous bone site with a closed growth plate (the distal tibial metaphysis, DTM) to ovariectomy (OVX) and OVX plus a prostaglandin E₂ (PGE₂) treatment, and compare the site's response to previous findings reported for another site (the proximal tibial metaphysis, PTM). Thirty-five 3-month-old female Sprague-Dawley rats were divided into five groups: basal, sham-OVX, and OVX+0, +1, or +6 mg PGE₂/kg/d injected subcutaneously for 3 months and given double fluorescent labels before sacrifice. Cancellous bone histomorphometric analyses were performed on 20- μ m-thick undecalcified DTM sections. Similar to the PTM, the DTM showed age-related decreases in bone formation and increases in bone resorption, but it differed in that at 3 months post-OVX, there was neither bone loss nor changes in formation endpoints. Giving 1 mg PGE₂/kg/d to OVX rats prevented most age-related changes and maintained the bone formation histomorphometry near basal levels. Treating OVX rats with 6 mg PGE₂/kg/d prevented age-related bone changes, added extra bone, and improved microanatomical structure by stimulating bone formation without altering bone resorption. Furthermore, after PGE₂ administration, the DTM, a cancellous bone site with a closed growth plate, increased bone formation more than did the cancellous bone in the PTM.

Key Words: Cancellous bone—Closed growth plate—Prostaglandin E₂—Bone formation—Bone resorption—Ovariectomized rats.

Introduction

Osteoporotic fractures in humans are limited to vertebrae, proximal femur and the wrist (Lindsay & Cosman 1992). In light of this, understanding the differences in bone site behavior among various animal species may lay the foundation for explaining site-specific differences in fracture rates. Despite the importance of this, studies of the behavior of different bone sites are limited.

Large animal and human studies are restricted to iliac crest biopsies (Podenphant & Engel 1987; Kimmel & Jee 1982).

In small animals, the ovariectomized-osteopenic rat model has been widely accepted for the study of the prevention and treatment of estrogen-deficient bone loss (Kalu et al. 1991; Wronski & Yen 1992). Most studies using this model have focused on the proximal tibial metaphysis (PTM) with fewer focusing on the distal femur metaphysis (DFM) and the lumbar vertebral body (LVB) (Kalu et al. 1991; Wronski & Yen 1992; Jee 1991c; Gasser & Jerome 1992; Wronski et al. 1989b). All these sites have nonfused growth plates.

To date, no studies have been performed on a metaphysis with a fully closed growth plate and low turnover rate. One such site in the rat is the distal tibial metaphysis (DTM), where the growth plate closes at 3 months (Dawson 1925). Recently, we described the histomorphometry of untreated and PGE₂-treated cancellous bone in the DTM in 7-13-month-old males. We found that the site contains low turnover cancellous bone and trabeculae similar in architecture to that seen in man. We also found that PGE₂ treatment induced more new bone formation in the DTM than in the PTM (Ke et al. 1993a; Ito et al. 1993). However, it still remained to be determined if the DTM would behave similarly in the estrogen-deficient (OVX) rat given PGE₂.

The current study is a continuation of a previous study by Ke et al. (1992c, 1993b) in which PGE₂ was used to prevent OVX-induced cancellous and endocortical bone loss. The cancellous and cortical bone sites analyzed were the PTM and the tibial shaft (TX). This report will deal with the following observations in the DTM: 1) the aging changes between 3 and 6 months; 2) the effects of OVX; 3) the responses to PGE₂ in the OVX rats; and 4) the comparative responsiveness of the DTM and the PTM to PGE₂ in OVX rats.

Materials and Methods

A complete description of the materials and methods we used was detailed in Ke et al. (1992c, 1993b). Briefly though, we divided 35 3-month-old virgin female Sprague-Dawley rats, weighing approximately 255 g (Charles River Laboratory, Inc., Portage, MI), into five groups. The first group was sacrificed at day 0 for basal controls. Group 2, used as aging controls, was sham-OVX and injected daily with a 20% ethanol vehicle for 90 days. Groups 3-5 were ovariectomized and simultaneously given

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vehicle (OVX + 0), 1 (OVX + 1) and 6 (OVX + 6) mg/kg/d prostaglandin E₂ subcutaneously for 90 days. There were 6–9 animals in each group. Powdered PGE₂ (Upjohn Co., Kalamazoo, MI) was prepared as previously reported (Ke et al. 1991). All rats received 25 mg/kg of tetracycline (achromycin-tetracycline hydrochloride; Lederle Laboratory, Pearl River, NY) on days 14 and 13 before sacrifice and 10 mg/kg of calcein (Sigma Chemical Co., St. Louis, MO) on days 4 and 3 before sacrifice. Except for the basal group, all rats also received a subcutaneous injection of 90 mg/kg xylenol orange (Fisher Scientific, Fairlawn, NJ) on day 0.

The rats were exsanguinated by heart puncture under ketamine anesthesia. The left tibia, lumbar vertebrae, femurs, serum and soft tissue were collected for future analyses. The right tibia was removed and defleshed. The distal tibia was cut at the tibiofibular junction, stained in Villanueva bone stain, and then processed to 100- μ m-thick longitudinal undecalcified sections for microradiography. The sections were further ground to 20 μ m for static and dynamic histomorphometric measurements (Jee et al. 1983; Ito et al. 1993).

Using a Video Image Analysis System and KSS Image Analysis Computer Programs, we determined total tissue area, trabecular bone area and perimeter. The measurements were taken from the area between the former epiphyseal–metaphyseal junction to 4 mm proximal to the junction. The percent trabecular bone area, trabecular width, number and separation were calculated from these measurements. The microanatomic trabecular bone structure indices were then measured. These indices consisted of the number of nodes, node to node, node to free end and

free end to free end. The measurements were then normalized to total tissue area and trabecular bone area in order to calculate their tissue- and bone-based densities (Garrahan et al. 1986; Compston et al. 1987, 1989; Ke et al. 1992c).

A digitizing image analysis system (DIAS) was used for the static and dynamic histomorphometric measurements of the 20- μ m sections of the distal tibial metaphyses. We used the same areas in the microradiographs for histomorphometric measurements. The parameters included total tissue area, trabecular bone area, perimeter and wall width, eroded perimeter, osteoid perimeter, single-labeled perimeter, double-labeled perimeter and interlabeling width. These parameters were used to calculate percent trabecular bone area, trabecular width, number and separation, as well as percent osteoid perimeter, percent eroded perimeter, percent labeled perimeter (double label + 1/2 label based), mineral appositional rate, bone formation rate-bone area and tissue area referent, formation period, resorption period, remodeling period, quiescent period and activation frequency (Frost 1976, 1977, 1981, 1983; Parfitt et al. 1987). We also determined the amount of newly formed cortical bone, which was found in the area between the xylenol orange label and the periosteal or endocortical surfaces (Figs. 8 and 9).

We evaluated the statistical differences between basal and other groups using the two-tailed Student's *t* test. The statistical differences between the sham-OVX and treatment groups were evaluated using ANOVA with Dunnett's *t* test (Neter & Wasserman 1982). A *Z* test (Nanivadekar & Kannappan 1990, 1991; Zhao et al. 1990; Niimoto et al. 1987) was employed to test whether the effects of PGE₂ in the DTM and PTM sites differed

Table 1. Comparison of proximal tibial (PTM) and distal tibial (DTM) cancellous bone: response to PGE₂ treatment (vs. basal)

| Parameters | Sites | Basal (mean \pm SD) | OVX + 6 mg treated (mean \pm SD) | OVX + 6 mg vs. basal (Z score) ^a | PGE ₂ effect vs. basal (<i>p</i> value) ^b |
|----------------------------------|-------|------------------------------|--|---|--|
| Trabecular area (%) | PTM | 15.9 \pm 2.4 | 21.4 \pm 7.1 | 2.3 \pm 2.9 | <i>p</i> < 0.05 |
| | DTM | 23.7 \pm 4.5 ^c | 45.2 \pm 13.2 | 4.8 \pm 2.9 | |
| Trabecular width (μ m) | PTM | 44 \pm 4 ^c | 57 \pm 9 | 3.5 \pm 2.4 | <i>p</i> < 0.01 |
| | DTM | 145 \pm 5 | 150 \pm 20 | 0.7 \pm 0.4 | |
| Trabecular number (#/mm) | PTM | 3.6 \pm 0.4 | 3.7 \pm 0.7 | 0.2 \pm 2.0 | NS |
| | DTM | 2.3 \pm 0.7 | 3.1 \pm 1.0 | 1.0 \pm 1.3 | |
| Trabecular separation (μ m) | PTM | 338 \pm 41 | 322 \pm 89 | -0.4 \pm 2.2 | NS |
| | DTM | 379 \pm 186 | 206 \pm 105 | -0.9 \pm 0.6 | |
| Labeled perimeter (%) | PTM | 17 \pm 2.9 ^c | 27.6 \pm 4.8 | 3.6 \pm 1.7 | <i>p</i> < 0.01 |
| | DTM | 15.4 \pm 8.2 ^c | 26.3 \pm 7.8 | 1.3 \pm 1.0 | |
| Eroded perimeter (%) | PTM | 7.9 \pm 0.9 ^c | 11.6 \pm 2.8 | 4.1 \pm 3.1 | <i>p</i> < 0.01 |
| | DTM | 2.6 \pm 1.5 | 2.1 \pm 0.8 | -0.3 \pm 0.5 | |
| Mineral apposition (μ m/d) | PTM | 1.1 \pm 0.2 | 1.3 \pm 0.3 | 1.6 \pm 1.8 | NS |
| | DTM | 0.7 \pm 0.2 | 0.9 \pm 0.2 | 1.0 \pm 1.1 | |
| Bone formation rate/BV (%/yr) | PTM | 258 \pm 76 | 405 \pm 145 | 1.9 \pm 1.9 | NS |
| | DTM | 59.0 \pm 25 | 94 \pm 38 | 1.4 \pm 1.5 | |
| Bone formation rate/TV (%/yr) | PTM | 40.5 \pm 11.1 ^c | 81.6 \pm 28.4 | 3.7 \pm 2.6 | NS |
| | DTM | 13.9 \pm 5.5 ^c | 43.4 \pm 21.6 | 5.3 \pm 3.9 | |
| Formation period (day) | PTM | 15.6 \pm 5.6 ^c | 23.2 \pm 7.4 | 1.4 \pm 1.3 | NS |
| | DTM | 17.0 \pm 4.6 ^c | 25.0 \pm 6.6 | 1.8 \pm 1.4 | |
| Resorption period (day) | PTM | 7.8 \pm 3.2 | 12.2 \pm 6.7 | 1.4 \pm 2.1 | NS |
| | DTM | 4.4 \pm 1.3 | 3.7 \pm 2.7 | -0.5 \pm 2.0 | |
| Remodeling period (day) | PTM | 23.4 \pm 8.6 ^c | 35.4 \pm 12.7 | 1.4 \pm 1.5 | NS |
| | DTM | 21.3 \pm 3.8 | 28.7 \pm 8.8 | 1.9 \pm 2.3 | |
| Activation frequency (cyc./yr) | PTM | 4.2 \pm 1.7 | 4.3 \pm 1.9 | 0.04 \pm 1.1 | NS |
| | DTM | 2.1 \pm 0.6 | 3.2 \pm 1.9 | 2.1 \pm 3.5 | |

^aZ score obtained from standardizing each group to an appropriate sets of controls and indicates how many standard changes from appropriate sets of controls. Basal will be zero.

^b*p* Value obtained from the *Z* test and indicates whether the effect of PGE₂ was different in DTM from PTM. A significant *P* value thus means that there is a significant difference in the effect of PGE₂ between the PTM and DTM.

^c*p* < 0.05 Vs. OVX + 6 mg PGE₂ group.

from the OVX-induced changes with basal-based Z scores (Table I) and whether they differed from the OVX-induced changes with OVX-based Z scores (Table II). All changes are significant at the $p < 0.05$ level unless noted otherwise.

Results

DTM changes when compared to basal (see Figs. 2–7).

Aging effects (sham-OVX). There were no bone mass alterations, but there was decreased trabecular bone width, node density, labeling perimeter, mineral apposition rate and bone formation rate-tissue and -bone levels and increased eroded perimeter and resorption and remodeling periods.

Ovariectomy effects (OVX+0). The OVX-induced changes were similar to those seen in the sham-OVX rats.

Ovariectomy plus 1 mg PGE₂/kg/d (OVX+1). This treatment mainly prevented age-related bone changes, that is, it maintained the bone histomorphometry at near basal levels. The exceptions were increases in remodeling period, formation period and a decrease in trabecular width, activation frequency and tissue level free end.

Ovariectomy plus 6 mg PGE₂/kg/d (OVX+6). This treatment prevented age-related bone changes, added trabecular bone and enhanced bone formation parameters. Both increases in trabecular area and in tissue-based bone formation rate were at the $p <$

0.01 level or less. Furthermore, free ends were lower and ratio of node to free end higher.

DTM changes when compared to 6-month-old (sham-OVX) and OVX (Figs. 2–7).

OVX+0. There were no significant differences between 6-month-old sham-OVX subjects and the vehicle treatment group.

OVX+1. Most bone formation parameters were higher (labeled perimeter, bone and tissue-based bone formation rates, trabecular wall width, formation period). However, cancellous bone mass was not higher and bone volume-based free end and ratio of node to free end were lower. Nevertheless, new metaphyseal cortical bone was seen with PGE₂ treatment (Fig. 8b).

OVX+6. The treated group had higher bone mass and better architecture. Trabecular area, width and tissue level node density were higher. Trabecular separation, bone- and tissue-level free ends were lower along with the stimulated bone formation end-points (labeled perimeter, mineral apposition rate, bone- and tissue-level bone formation rates, activation frequency). There was less eroded perimeter and a shortened resorption period when compared to OVX rats. The bone volume-based node density was also lower. Higher trabecular area, width, ratio of node to free end and bone formation parameters and lower trabecular separation and eroded perimeters were significant at the $p < 0.01$ level or less.

Table II. Comparison of proximal tibial (PTM) and distal tibial (DTM) cancellous bone response to PGE₂ treatment (vs. OVX)

| Parameters | Sites | OVX + 0 mg (mean ± SD) | OVX + 6 mg treated (mean ± SD) | OVX + 6 mg vs. OVX/0 (Z score) ^a | PGE ₂ effect vs. OVX/0 (p value) ^b |
|---------------------------------|-------|---------------------------|--------------------------------------|---|--|
| Trabecular area (%) | PTM | 3.0 ± 1.8 ^c | 21.4 ± 7.1 | 10.1 ± 3.9 | NS |
| | DTM | 20.8 ± 3.3 ^c | 45.2 ± 13.2 | 7.4 ± 4.0 | |
| Trabecular width (μm) | PTM | 36 ± 8 ^c | 57 ± 9 | 2.6 ± 1.1 | $p < 0.01$ |
| | DTM | 92 ± 6 ^c | 150 ± 20 | 9.8 ± 3.3 | |
| Trabecular number (#/mm) | PTM | 0.8 ± 0.5 ^c | 3.7 ± 0.71 | 5.8 ± 1.4 | NS |
| | DTM | 2.3 ± 0.2 | 3.1 ± 1.0 | 3.3 ± 4.0 | |
| Trabecular separation (μm) | PTM | 3703 ± 4930 ^c | 322 ± 89 | -2.7 ± 0.0 | $p < 0.01$ |
| | DTM | 354 ± 50 ^c | 206 ± 105 | -1.0 ± 2.1 | |
| Labeled perimeter (%) | PTM | 24.6 ± 5.3 | 27.6 ± 4.8 | 0.6 ± 0.9 | $p < 0.01$ |
| | DTM | 4.5 ± 1.3 ^c | 26.3 ± 7.8 | 17.0 ± 6.1 | |
| Eroded perimeter (%) | PTM | 16.2 ± 6.9 | 11.6 ± 2.8 | -0.7 ± 0.4 | NS |
| | DTM | 4.6 ± 1.4 | 2.1 ± 0.8 | -0.9 ± 0.2 | |
| Mineral apposition rate (μm/d) | PTM | 0.9 ± 0.1 ^c | 1.3 ± 0.3 | 4.4 ± 2.9 | $p < 0.01$ |
| | DTM | 0.6 ± 0.1 ^c | 0.9 ± 0.2 | 1.4 ± 0.9 | |
| Bone formation rate/BV (%/yr) | PTM | 376 ± 87.4 | 405 ± 145 | 0.3 ± 1.7 | $p < 0.05$ |
| | DTM | 18.5 ± 9.4 ^c | 94 ± 38 | 8.1 ± 4.0 | |
| Bone formation rate/TV (%/yr) | PTM | 10.9 ± 6.8 ^c | 81.6 ± 28.4 | 10.5 ± 4.2 | $p < 0.01$ |
| | DTM | 3.7 ± 1.6 ^c | 43.4 ± 21.6 | 24.8 ± 13.5 | |
| Formation period (day) | PTM | 12.8 ± 1.2 ^c | 23.2 ± 7.4 | 2.4 ± 1.0 | NS |
| | DTM | 16.3 ± 6.2 ^c | 25.0 ± 6.6 | 1.4 ± 1.1 | |
| Resorption period (day) | PTM | 4.8 ± 2.2 ^c | 12.2 ± 6.7 | 3.3 ± 2.3 | $p < 0.01$ |
| | DTM | 27.3 ± 41.2 ^c | 3.7 ± 2.7 | -0.6 ± 0.1 | |
| Remodeling period (day) | PTM | 17.7 ± 1.0 ^c | 35.4 ± 12.7 | 2.7 ± 1.3 | $p < 0.01$ |
| | DTM | 43.6 ± 46 | 28.7 ± 8.8 | -0.3 ± 0.2 | |
| Activation frequency (cycle/yr) | PTM | 9.1 ± 0.9 ^c | 4.3 ± 1.9 | -0.1 ± 0.4 | $p < 0.01$ |
| | DTM | 1.3 ± 0.9 ^c | 3.2 ± 1.9 | 2.3 ± 2.2 | |

^aZ score obtained from standardizing each group to an appropriate sets of controls and indicates how many standard changes from appropriate sets of controls. OVX will be zero.

^bp value obtained from the Z test and indicates whether the effect of PGE₂ was different in DTM from PTM. A significant p value thus means that there is a significant difference in the effect of PGE₂ between the PTM and DTM.

^c $p < 0.05$ Vs. OVX + 6 mg PGE₂ group.

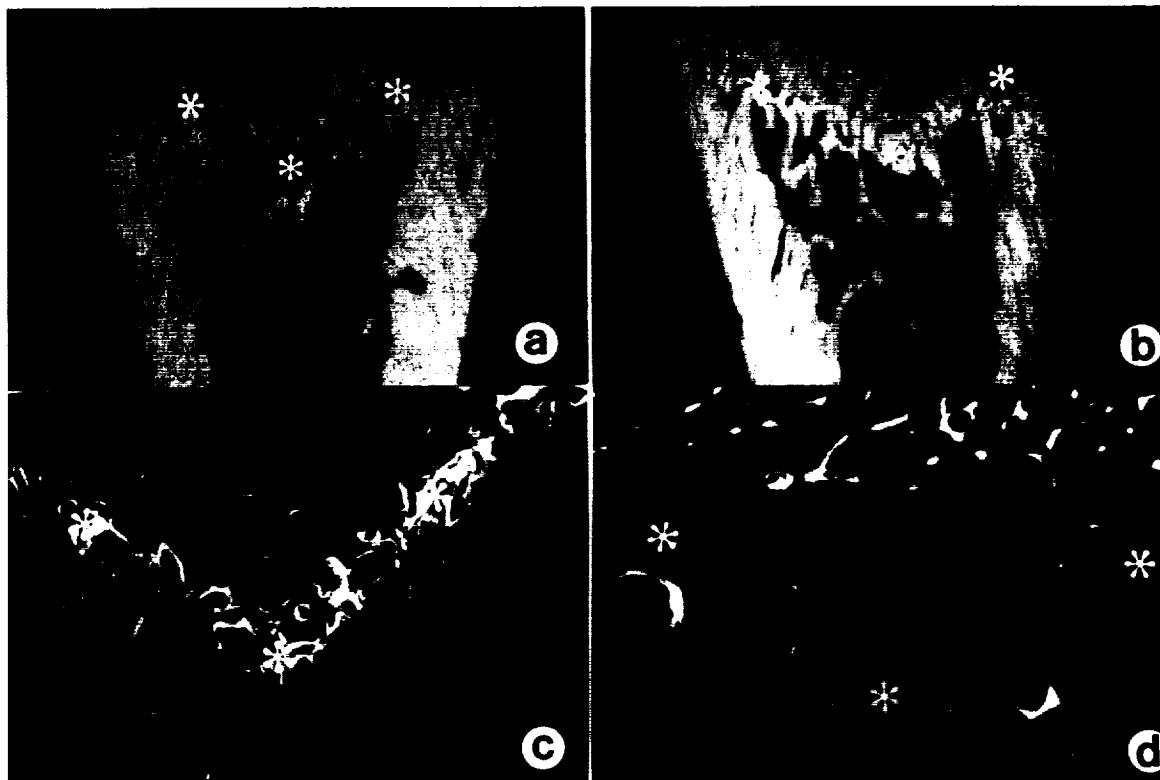


Fig. 1. Microradiographs and fluorescent micrographs showing a partially closed growth plate (a) with its fluorescent labeling (c) in a 3-month-old DTM and a closed growth plate (b) with reduced fluorescent labeling (d) in a 6 month old DTM. Asterisk indicates former epiphyseal-metaphyseal junction; (a) and (b): $\times 20$, (c) and (d): $\times 60$.

Comparison of OVX + PGE₂-induced changes in the DTM and PTM (Tables I and II)

Tables I and II show the different responses to PGE₂ treatment at the DTM and PTM (Ke et al. 1992c) in the same animals. For many parameters, the DTM site responded more favorably to PGE₂ treatment than the PTM site. When compared to the basal or pretreatment controls, PGE₂ treatment did not induce bone mass change in the PTM ($15.9 \pm 2.4\%$ to $21.4 \pm 7.1\%$; $p > 0.05$); however, it did add 91% new bone in the DTM ($23.7 \pm$

4.5% to $45.2 \pm 13.2\%$; $p < 0.01$). Furthermore, basal control-based Z scores for bone mass were higher in the DTM than in the PTM (4.8 ± 2.9 vs. 2.3 ± 2.9 ; $p < 0.05$); however, increases in trabecular width and labeled and eroded perimeters were higher in the PTM (Table I).

In contrast to the above, several parameters improved more in the PTM than in the DTM after treatment. When compared to changes in the OVX controls, PGE₂ treatment resulted in 600% more bone in the PTM (3.0 ± 1.8 to $21.4 \pm 7.1\%$), but only 87% more in the DTM ($20.8 \pm 3.3\%$ to $45.2 \pm 13.2\%$). How-

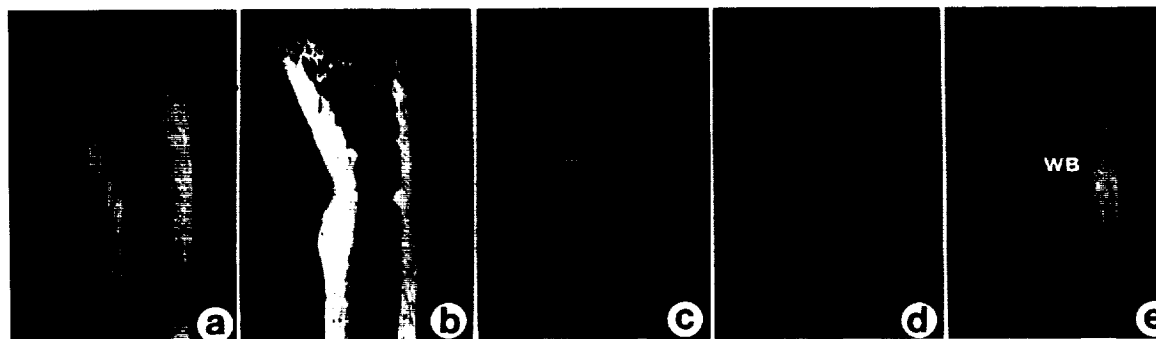


Fig. 2. Microradiographs showing cancellous bone changes in DTM from basal (a), sham-OVX (b), OVX controls (c), and OVX rats treated with 1 mg (d) and 6 mg (e) PGE₂/kg/d for 90 days. There were no obvious differences in bone mass and other microanatomic structures among basal (a), sham-OVX (b) and OVX controls (c). Metaphyseal cancellous bone mass was slightly greater in 1 mg PGE₂/kg/d-treated OVX rats (d) than in basal (a), sham-OVX (b) and OVX controls (c). More cancellous bone mass, thicker cortex, and added trabecular bone (WB) were found in the 6-mg PGE₂-treated OVX rats. One-hundred-micron microradiography section. ($\times 6$).

ever, the OVX control-based Z scores for bone mass were not significantly different (7.4 ± 4.0 in the DTM vs. 10.1 ± 3.9 in the PTM). Moreover, other OVX-based Z scores showed the trabecular width, labeled perimeter and bone formation rates increased more after PGE₂ treatment in the DTM than in the PTM, while trabecular separation decreased and mineral apposition rate increased more in the PTM than in the DTM (Table II).

Discussion

Aging had only a limited effect on the cancellous bone in the DTM site between 3- and 6-month-old rats. It reduced the trabecular bone width, bone formation parameters (labeled perim-

eter, mineral apposition rate and bone formation rate), and increased the eroded perimeter and the resorption and remodeling periods. This resulted in histomorphometric values for the 6-month-old DTM sites similar to those we previously reported in 7-month-old males (Ito et al. 1993). These changes in bone resorption and formation activities were not surprising, since at 3 months the DTM was in the process of closing the epiphysis and transforming its primary spongiosa into secondary spongiosa (Fig. 1).

Our study showed that there had been no bone mass lost nor had there been any architectural changes 3 months after OVX in the DTM. There are several possible explanations for the lack of estrogen-deficiency-induced bone loss. One is the extremely low bone turnover rate of the DTM. There is a strong relationship

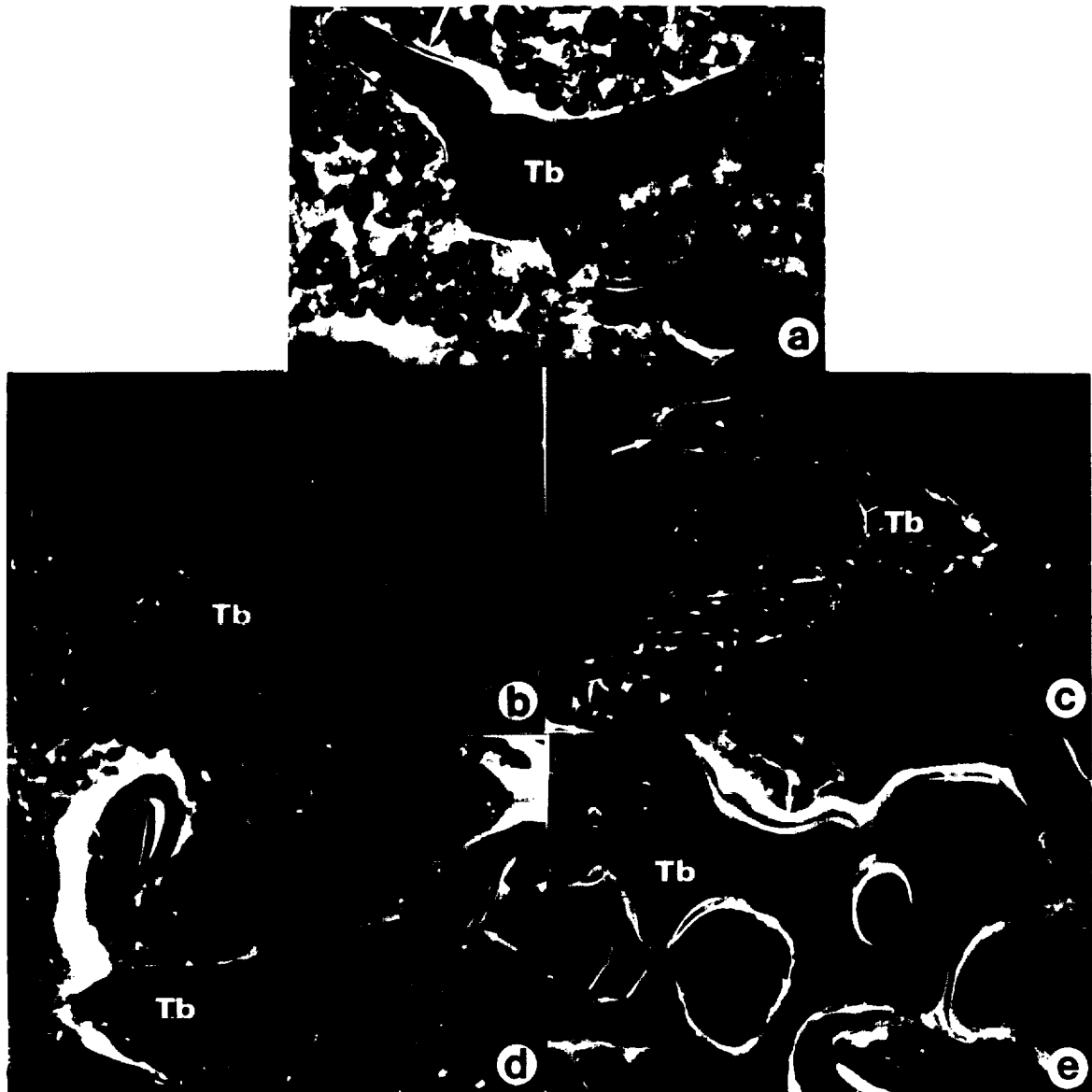


Fig. 3. The fluorescent micrographs of DTM cancellous bone from basal (a), sham-OVX (b), OVX control (c), and OVX rats treated with 1 (d) and 6 (e) mg PGE₂ for 3 months. A dramatic reduction in labelling surface was observed in sham-OVX [(b) 6-month-old] compared to those of basal [(a) 3-month-old] controls. OVX + 1 mg PGE₂/kg/d prevented this reduction (d), while OVX + 6 mg PGE₂/kg/d showed increased interlabeling width and labeling surfaces (e). Tb = trabeculae; arrows = double labeled surfaces. Twenty-micron sections ($\times 150$).

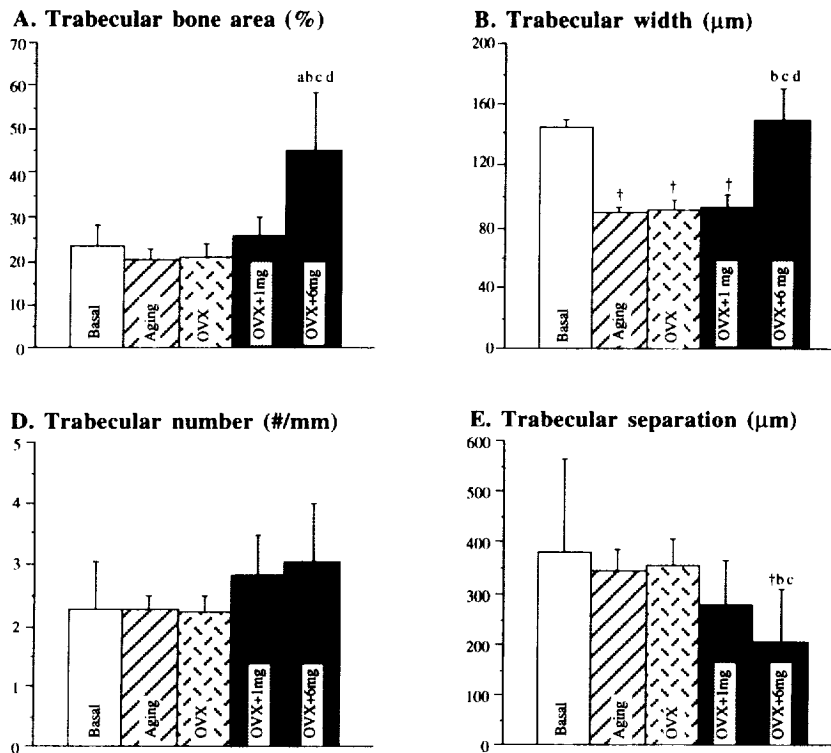


Fig. 4. Static histomorphometric indices of DTM cancellous bone. (†) $p < 0.05$, $p < 0.01$ vs. basal controls; (*) $p < 0.05$, $p < 0.01$ vs. sham-OVX controls; (#) $p < 0.05$, $p < 0.01$ vs. OVX controls; (@) $p < 0.05$, $p < 0.01$ vs. OVX + 1 mg PGE₂ (mean \pm SD). OVX and OVX + 1 mg PGE₂/kg/d treatment induced no difference from sham-OVX controls. OVX + 6 mg PGE₂/kg/d increased trabecular area (A), width (B) and decreased trabecular separation (E) from OVX controls.

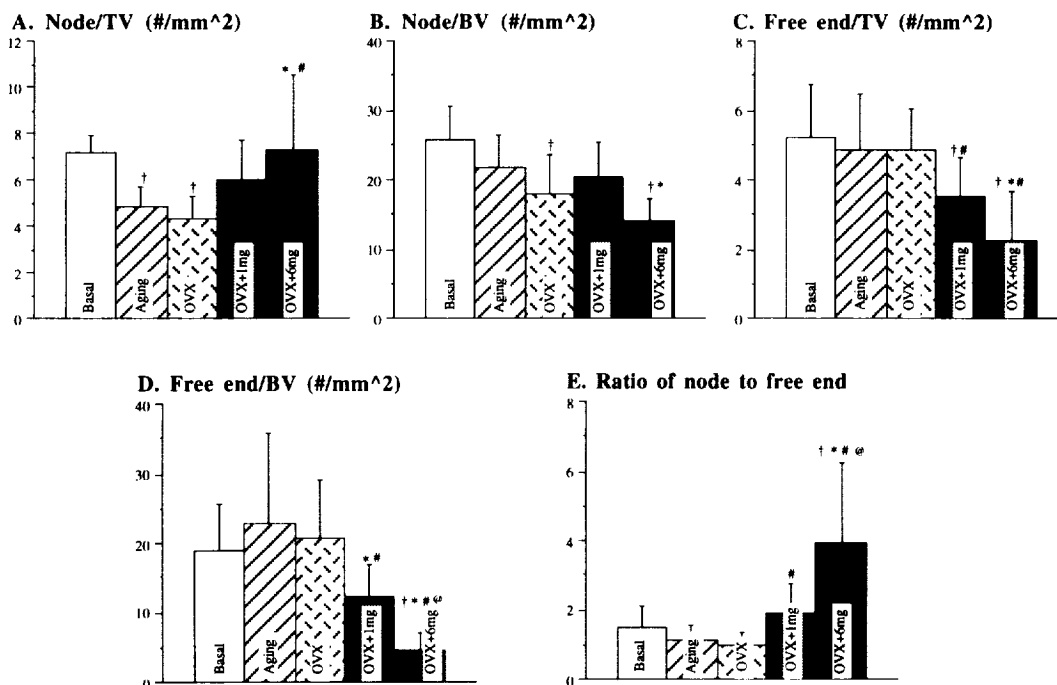


Fig. 5. Microanatomical indices of the DTM cancellous bone. (†) $p < 0.05$ vs. basal controls; (*) $p < 0.05$ vs. sham-OVX controls; (#) $p < 0.05$ vs. OVX controls; (@) $p < 0.05$ vs. OVX + 1 mg PGE₂ (mean \pm SD). OVX did not induce any difference from sham-OVX controls. OVX + 1 mg PGE₂/kg/d decreased free end density (C and D) and increased ratio of node to free end (E), while OVX + 6 mg PGE₂/kg/d increased tissue-level node density (A).

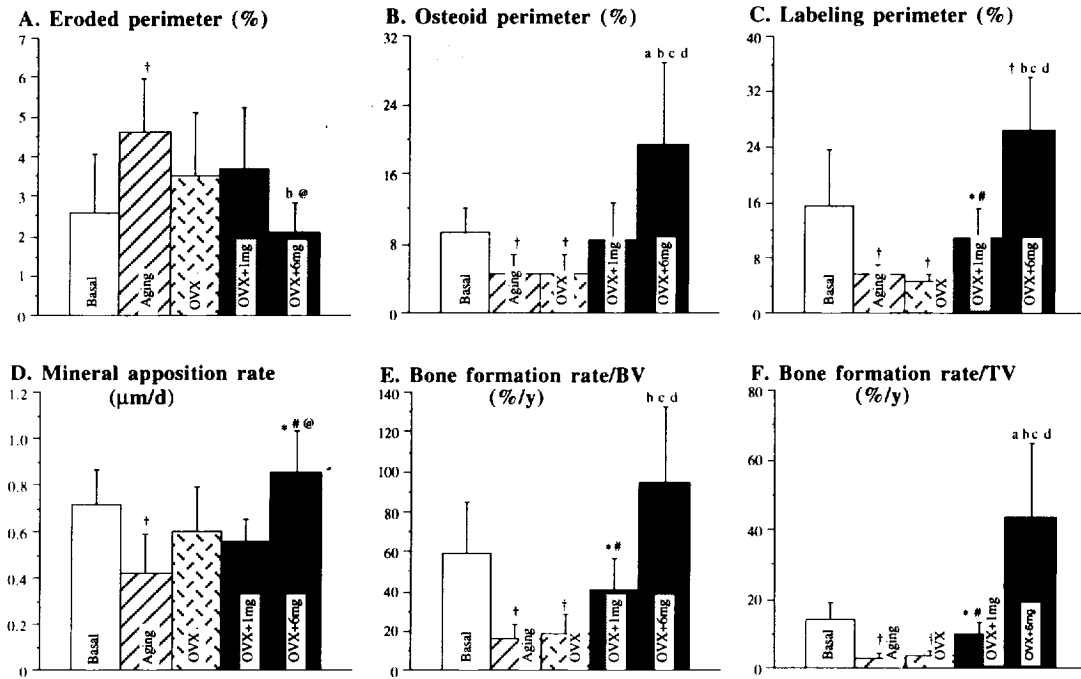


Fig. 6. Dynamic histomorphometric indices of the distal tibial metaphyseal cancellous bone. (†) $p < 0.05$, $p < 0.01$ vs. basal controls; (*) $p < 0.05$, $p < 0.01$ vs. sham-OVX controls; (#) $p < 0.05$, $p < 0.01$ vs. OVX controls; (@) $p < 0.05$, $p < 0.01$ vs. OVX + 1 mg PGE₂ (mean ± SD). There was decreased bone formation (B–F) and increased bone resorption (A) between 3 and 6 months of age. OVX did not cause any difference from sham-OVX controls (A–F). OVX + 1 mg PGE₂/kg/d prevented age-related bone formation decrease (B–F). OVX + 6 mg PGE₂/kg/d increased bone formation (B–F) and decreased bone resorption (A).

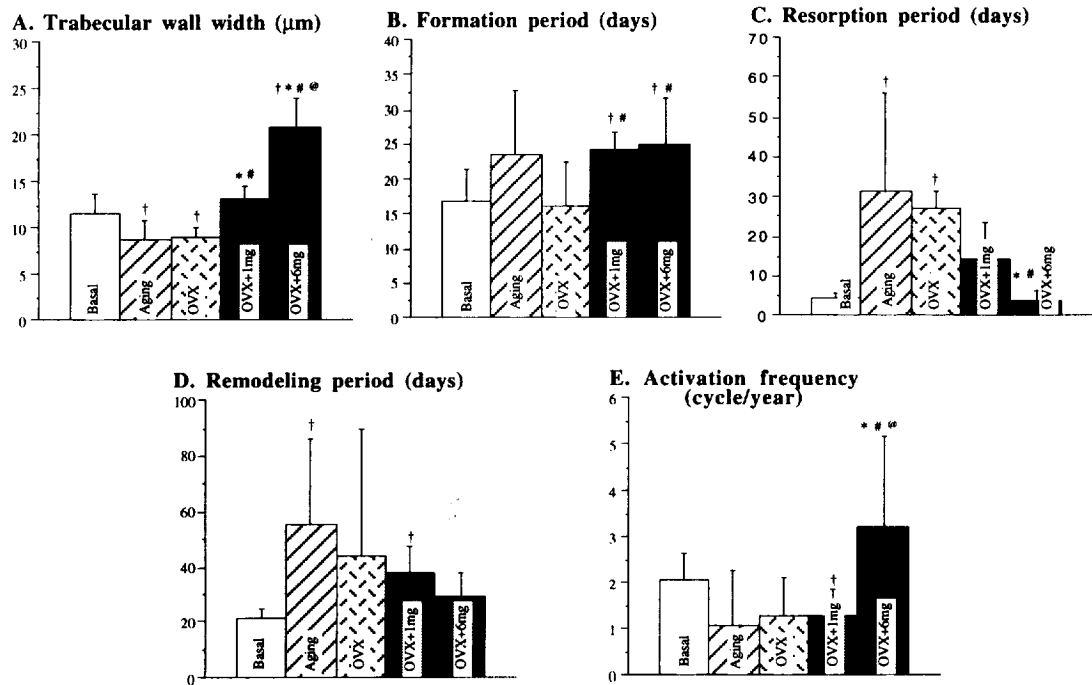


Fig. 7. Trabecular wall width, formation, resorption, remodeling periods and activation frequency of the DTM cancellous bone. (†) $p < 0.05$ vs. basal controls; (*) $p < 0.05$ vs. sham-OVX controls; (#) $p < 0.05$ vs. OVX controls; (@) $p < 0.05$ vs. OVX + 1 mg PGE₂ (mean ± SD). OVX did not cause any difference from sham-OVX controls (A–E). OVX + 1 mg PGE₂/kg/d increased trabecular wall width (A) and prolonged formation period (B) from OVX controls. OVX + 6 mg PGE₂/kg/d increased trabecular wall width (A), activation frequency (E), formation period (B) and shortened resorption period (D).

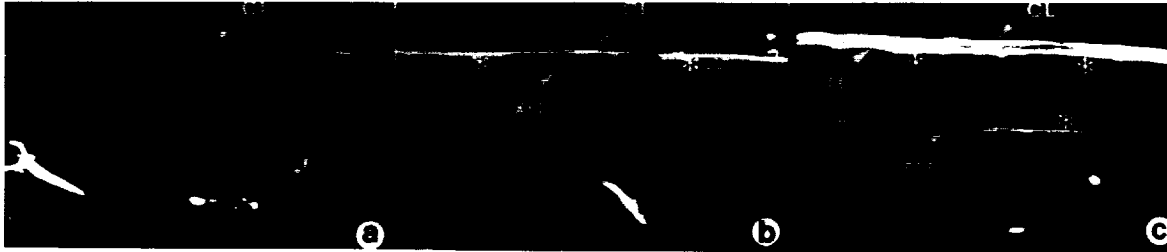


Fig. 8. Fluorescent micrographs of a portion of periosteal surface of DTM from OVX (a) and OVX rats treated with 1 mg (b) and 6 mg (c) PGE₂ for 90 days. More newly formed periosteal bone (*) was observed in OVX + 1 mg (b) and 6 mg (c) PGE₂/kg/day-treated subjects than in OVX-treated subjects (a). XO = xylenol orange label; CL = calcein label; TC = tetracycline label. Twenty-micron sections ($\times 80$).

between OVX-induced bone loss and bone turnover rates in the rat, in that OVX-induced cancellous bone loss is greater in the PTM than in the LVB metaphyses (Wronski et al. 1988, 1989a,b), and the bone turnover is much more rapid in the PTM (Li et al. 1991). The second explanation pertains to the differences in mechanical loading. It is conceivable that the DTM is more heavily loaded than the PTM because, with its closed growth plate, it lacks a cartilaginous shock absorber to absorb some of the mechanical loading and, being situated farther back in the skeleton, it may bear more body weight. Overloading tends to stimulate bone formation and depress bone resorption (Frost 1964, 1988a,b; Jee et al. 1990, 1991a,b); thus, the low bone turnover rate in the DTM may be due to heavy loading and, in turn, be responsible for the lack of OVX-induced bone loss.

Although the use of a 1 mg PGE₂ treatment on OVX rats did not increase cancellous bone mass, it did add new cortical bone and increase bone formation parameters which improved architecture. A longer treatment period may add extra bone. The 6-mg PGE₂ treatment of OVX rats prevented age-related bone changes, added extra bone, and improved the microanatomical structure appreciably by stimulating bone formation. This is in agreement with our previous findings. We have demonstrated that this dose will add cancellous and cortical bone to intact, OVX and immobilized (IM) limbs (Ke 1992a,c, 1993b; Akamine 1992), as well as restore bone loss associated with OVX and IM (Mori et al. 1992; Tang et al. 1992; Li et al. 1993). Both doses of PGE₂ added periosteal as well as endocortical bone mass to the DTM (Figs. 8 and 9). In an earlier report, we noted that this treatment added extra bone in tibial diaphyses in intact, OVX and IM rats (Jee et al. 1991b,c, 1992a; Tang et al. 1992).

We decided when comparing the responsiveness of the DTM and PTM to the ability of PGE₂ to add bone in OVX rats, to base comparisons on basal control values rather than on those of the OVX control. Since this is a prevention study, the PGE₂ treat-

ment did not allow OVX-induced bone loss in the PTM, but instead it maintained bone in the PTM (pretreatment $15.9\% \pm 2.4\%$, final $21.4 \pm 7.1\%$; $p > 0.05$) and added more bone to the DTM (pretreatment $23.7 \pm 4.5\%$, final $45.2 \pm 13.2\%$; $p < 0.01$). Thus, it was more realistic to compare the PGE₂ effects to pretreatment or basal controls. Nevertheless, it was useful to determine an OVX-control-based Z score because it tested the responses of the two sites where OVX had had no effect on the DTM bone mass but had induced bone loss in the PTM. The OVX-based Z score did not show any significant difference between increases in bone mass in the DTM and those in the PTM. However, most of the bone formation endpoints (increased width, labeled perimeter, bone formation rates and activation frequency and shortened resorption and remodeling periods; Table II) were higher in the DTM. The DTM is more responsive than the PTM to PGE₂'s ability to induce a positive bone balance. The same conclusion was reached in an earlier study comparing the response of PGE₂-induced bone formation in intact male Sprague-Dawley rats (Ito et al. 1993; Ke et al. 1993a).

The fact that no cancellous bone loss was induced in the 3 months post-OVX in the DTM (a cancellous bone site with a closed growth plate) suggests that bone is not lost at a uniform rate in all skeleton sites after menopause. Some sites may not lose any bone after OVX; however, a longer-term study is needed to confirm this. Finding variable rates of bone loss in rats may be analogous to finding different fracture rates in humans. This is borne out in humans where osteoporotic fractures are seen predominantly in the vertebral column, distal forearm (Colles) and the hip. In addition, the finding that PGE₂ can add bone to low turnover cancellous bone sites indicates that bone anabolic agents will be effective in senile (Type II) osteoporosis (i.e., low turnover osteoporosis). It also suggests the presence of osteoprogenitor cells capable of responding to PGE₂ in the DTM and, perhaps, other fatty marrow sites. In this regard, the rat DTM can be used to advantage as another site for studying the effects of such anabolic agents on bone.



Fig. 9. Fluorescent micrographs of a portion of endocortical surface of DTM from OVX control (a), and OVX rats treated with 1 mg (b) and 6 mg (c) PGE₂ for 90 days. Newly formed endocortical bone (*) was observed only in the OVX + 1 mg and 6 mg PGE₂/kg/d treated rats (b and c). XO = xylenol orange label; CL = calcein label; TC = tetracycline label. Twenty-micron sections ($\times 80$).

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