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Prostaglandin E_2 prevents ovariectomy-induced cancellous bone loss in rats

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Summary

The object of this study was to determine whether prostaglandin E_2 (PGE_2) can prevent ovariectomy-induced cancellous bone loss. Thirty-five 3-month-old female Sprague-Dawley rats were divided into two groups. The rats in the first group were ovariectomized (OVX) while the others received sham operation (sham-OVX). The OVX group was further divided into three treatment groups. The daily doses for the three groups were 0, 1 and 6 mg PGE_2/kg for 90 days. Bone histomorphometric analyses were performed on double-fluorescent-labeled undecalcified proximal tibial metaphysis (PTM). We confirmed that OVX induces massive cancellous bone loss (−80%) and a higher bone turnover (+143%). The new findings from the present study demonstrate that bone loss due to ovarian hormone deficiency can be prevented by a low-dose (1 mg) daily administration of PGE_2. Furthermore, a higher-dose (6 mg) daily administration of PGE_2 not only prevents bone loss but also adds extra bone to the proximal tibial metaphyses. PGE_2 at the 1-mg dose level significantly increased trabecular bone area, trabecular width, trabecular node density, density of node to node, ratio of node to free end, and thus significantly decreased trabecular separation from OVX controls. At this dose level, these same parameters did not differ significantly from sham-OVX controls. However, at the 6-mg dose level PGE_2, there were significant increases in trabecular bone area, trabecular width, trabecular node density, density of node to node, and ratio of node to free end, while there was significant decrease in trabecular separation from both OVX and sham-operated controls. The changes in indices of trabecular bone microanatomical structure indicated that PGE_2 prevented bone loss as well as the disconnection of existing trabeculae. In summary, PGE_2 administration to OVX rats decreased bone turnover and increased bone formation parameters resulting in a positive bone balance that prevented bone loss (in both lower and higher doses) and added extra bone to metaphyses of OVX rats (in higher dose). These findings support the strategy of the use of bone stimulation agents in the prevention of estrogen depletion bone loss (postmenopausal osteoporosis).

Key words: Prostaglandin E_2; Prevention; Ovariectomy; Cancellous bone; Architecture; Bone remodeling

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Introduction

The positive bone balance (anabolic) effects of exogenous prostaglandin E_2 (PGE_2) on bone are well known: daily administration of PGE_2 can increase bone mass in both animals [1–12] and man [13–15].

Previously, we reported that exogenous PGE_2 not only increased bone mass in intact male and female rats by activating bone modeling and remodeling in favor of bone formation [16–22], but also restored bone to osteopenic ovariectomized (OVX) rats [16–18]. In light of these facts, we postulated that PGE_2 will prevent ovariectomy-induced bone loss if given immediately following ovariectomy. The present study tested this postulate. It used a 90-day experimental period to allow both bone loss in OVX rats and bone gain in PGE_2-treated rats to reach a new steady state [19,23]. This period equals at least two to three bone remodeling cycles [19,24].

Materials and Methods

Thirty-five 3-months-old virgin female Sprague-Dawley rats, weighing approximately 255 g (Charles River Laboratory, Inc., Portage, MI) were acclimated to local vivarium conditions (24°C and 12 h/12 h light–dark cycle) for 7 days. During the experimental period, each animal was housed in a separate plastic module (21 × 32 × 20 cm) and allowed free access to water and a pelleted (commercial) natural-product diet (Rodent Laboratory Chow 5001, Ralston-Purina Co., St. Louis, MO), which contained 1.46% of calcium, 0.99% of phosphorus and 4.96 IU/g of Vit. D_3. The rats were divided into five groups (6–9 rats per group). The first group (six rats) was sacrificed at day 0 for baseline or basal controls. Group 2 (six rats) was sham-OVX and treated simultaneously with a vehicle injection for 90 days. Groups 3–5 were OVX and treated simultaneously with 0 (six rats), 1 (eight rats) and 6 (nine rats) mg PGE_2/kg/day, respectively, for 90 days.

All rats, except those killed at day 0, received daily injections of 1 ml/kg PGE_2 or a vehicle solution. Powdered PGE_2 (The Upjohn Co., Kalamazoo, MI) was prepared as previously [19]. Groups 2 (sham-OVX controls) and 3 (OVX controls) received a vehicle (20% ethanol) while group 4 (OVX + 1 mg/kg/day) received 1 mg/ml, and group 5 (OVX + 6 mg/kg/day) received 6 mg/ml daily subcutaneously on the back [19].

All rats received a subcutaneous injection of 25 mg/kg of tetracycline (Achomycin–tetracycline hydrochloride; Lederle Laboratory, Pearl River, NY) on the 14th and 13th day and 10 mg/kg of calcein (Sigma Chemical Co., St. Louis, MO) on the 4th and 3rd day before sacrifice.

At autopsy, all rats were anesthetized by intraperitoneally injecting a mixed solution of 50 mg/kg of ketamine hydrochloride (Veterinary Products, Bristol Laboratories, Div. of Bristol-Myers Co., Syracuse, NY) and 10 mg/kg of xylazine (Mobay Corporation, Animal Health Division, Shawnee, KS) at 1 ml/kg body weight. The rats were exsanguinated by heart puncture and the serum was stored

frozen, but was not analyzed because there were numerous publications on the subject [11,16,19,25,26]. The lungs, liver, adrenal glands, spleen, kidneys and thymus were removed and weighed. These organ weights were normalized to body weight as follows: mean body weight of all rats divided by body weight of each rat times organ weight.

The left tibiae were removed and stored in 70% ethanol for the measurement of tibial length. The length of left tibia was measured using a Boley Millimeter Caliper Gauge (The L.S. Starrett Co., Athol, MA).

The right tibia was removed, dissected and cut into three equal parts. The proximal third was processed to 100 μm and then microradiographed. Thereafter sections were further ground to a thickness of 20 μm and coverslipped for morphometric measurements [16,19–21,25–28].

Using a Video Image Analysis System and KSS Image Analysis, we measured total tissue area, trabecular bone area and perimeter to calculate percent trabecular bone area, trabecular width, number and separation [29] in microradiographs of proximal tibial metaphyseal area between 1 and 4 mm distal to the growth plate–metaphyseal junction [30]. We also measured the microanatomic trabecular bone structural indices which were previously defined by Garrahan et al. [31–34]. The indices included: number of nodes, node to node, node to free end, cortex to node, free end to free end and cortex to free end. These numbers were normalized to total tissue area and trabecular bone area in order to calculate their tissue- and bone-based densities. These indices provided data on the interconnectedness of the trabecular bone structure and on the number of structural elements. Node to node and cortex to node indicated interconnectedness, while free end to free end, node to free end and cortex to free end represented a lack of interconnectedness. When the trabeculae or struts were connected, the node to free end ratio was high, and low when the structures were broken [31–34].

A digitizing image analysis system (DIAS) was used for the static and dynamic histomorphometric measurements of the proximal tibial metaphyseal area between 1 and 4 mm distal to the growth plate–metaphyseal junction (same as in microradiographs). The parameters included total tissue area, trabecular bone area and perimeter, eroded perimeter, osteoid perimeter, and trabecular wall width, single-labeled perimeter, double-labeled perimeter, and interlabel width (at the trabecular surface and the growth plate-metaphyseal junction region). These parameters were used to calculate percent trabecular bone area, trabecular width, number and separation [30], as well as percent osteoid perimeter, percent eroded perimeter, percent labeled perimeter, mineral apposition rate, bone formation rate–bone area and tissue area referent, formation period, resorption period, remodeling period, quiescent period, activation frequency and longitudinal growth rate [16,17,19–21,25–29,35]. We included an additional observation and measurement, the frequency and the amount of diffusely labeled trabecular bone, which has been defined previously [18]. Furthermore, we confirmed the presence of woven bone by the irregular orientation of osteocytes and collagen bundles by bright field and polarized light microscopes [36]. This parameter represents woven bone formed at 14, 13 and 4, 3 days before sacrifice when

fluorescent markers were injected, and it underestimates the total amount of woven bone [18]. We estimated the mean accumulated longitudinal growth as follows: $((LGR_{basal} + LGR_{final})/2) \times 90$ days (newly generated metaphysis during the study period).

Statistical differences between basal controls and other groups were evaluated using the two-tail Student *t*-test. The statistical differences between age-matched control and treatment groups were evaluated using ANOVA with Dunnett's *t*-test [37].

Results

Effects on body weight

Figure 1 shows changes in body weight with time. Compared to sham controls, the body weight of OVX control rats began to increase 20 days post OVX and continued increasing until 60 days post OVX, then plateaued thereafter. This finding is consistent with findings by others [38–40,46,49]. In OVX rats treated with 1 mg PGE_2/kg/day, body weight increased by 12 to 18% from sham controls but was unchanged from OVX controls, while in OVX rats treated with 6 mg/kg/day, body weight increased compared to sham controls (8 to 10%), but decreased compared to OVX controls (6 to 12%).

Effects on soft tissue weights

Figure 2 shows the changes in soft tissue weights after normalization to body weight. Compared to basal controls, spleen weight decreased (8%) in sham controls. Compared to sham controls, OVX control rats exhibited decreased liver and lung weights (8 and 9%), and increased spleen and thymus weights (14 and 55%). In PGE_2-treated OVX rats (1 and 6 mg/kg/day), there was an

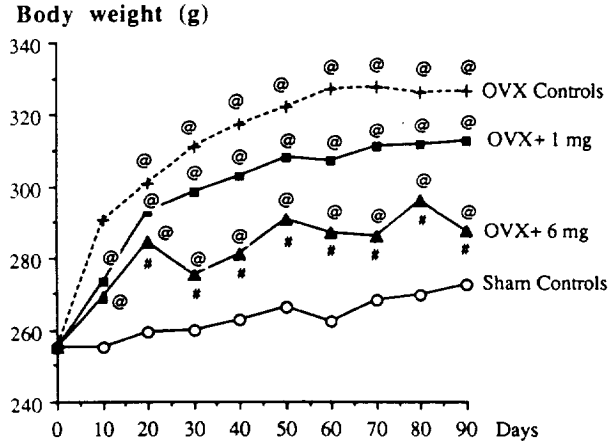

Fig. 1. Time course of body weight changes in sham-operated controls, OVX controls and PGE_2-treated OVX rats. $^{@}P<0.05$ vs. sham-operated controls; $^{\#}P<0.05$ vs. OVX controls; $^{*}P<0.05$ vs. OVX + 1 mg.

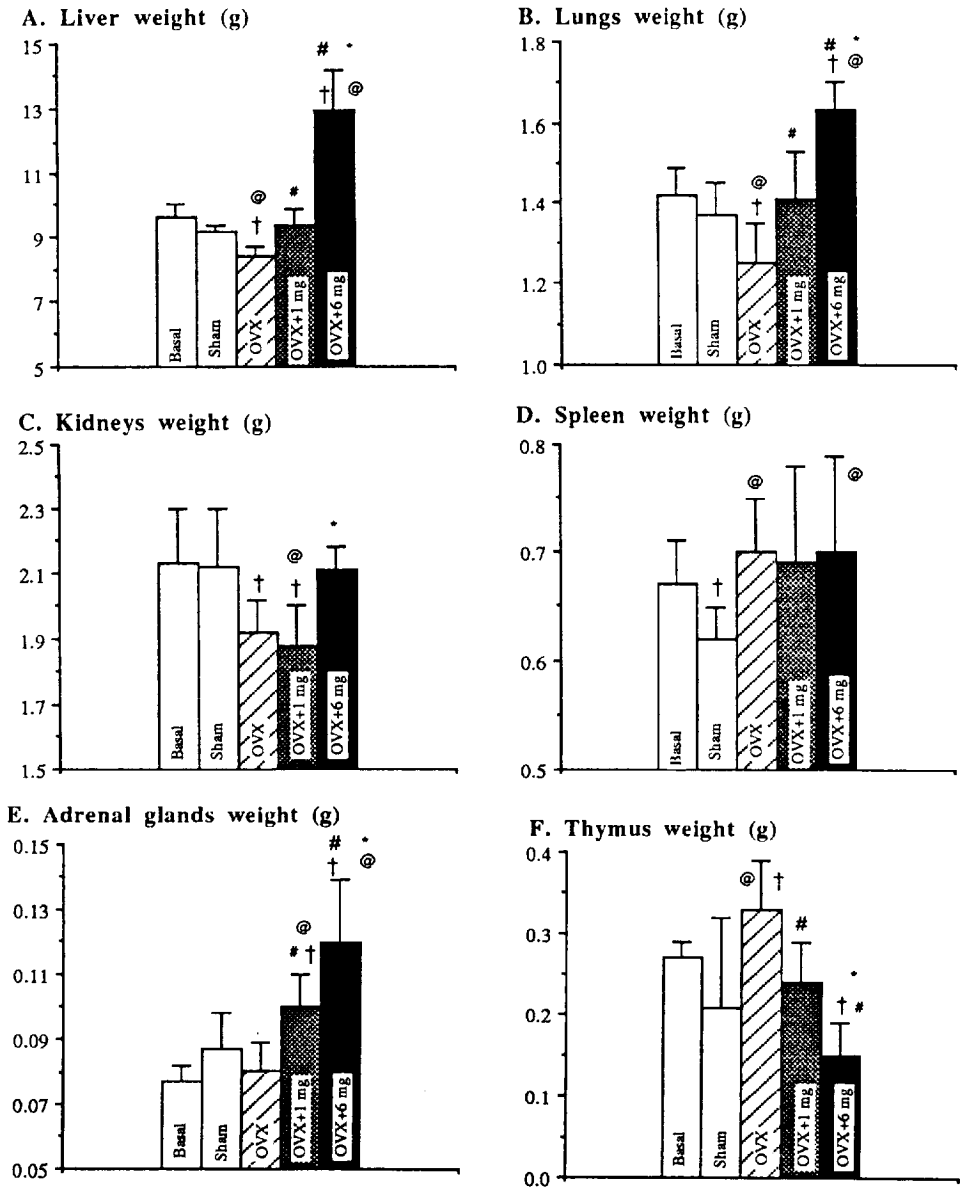

Fig. 2. Organ weight changes in basal controls, sham-operated controls, OVX controls and PGE_2-treated OVX rats. Y error bar represents standard deviation. $^{\dagger}P<0.05$ vs. basal controls; $^{@}P<0.05$ vs. sham-operated controls; $^{\#}P<0.05$ vs. OVX controls; $^{*}P<0.05$ vs. OVX + 1 mg.

increase in weight of liver (12 and 55%), lungs (13 and 31%), adrenal glands (27 and 53%), and a decrease in weight of thymus (26 and 54%) compared to OVX controls. In the OVX rats treated with 1 mg PGE_2/kg/day, kidney weight decreased (11%) while adrenal gland weight increased (15%) when compared to those of sham controls. In the OVX rats treated with 6 mg PGE_2/kg/day,

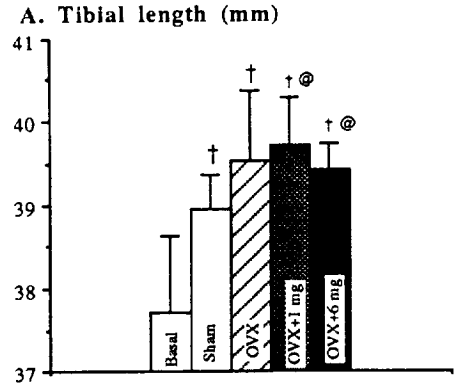

Fig. 3. Effects on tibial length in basal controls, sham-operated controls, ovariectomized controls and PGE_2-treated ovariectomized rats. Y error bar represents standard deviation. $^{\dagger}P<0.05$ vs. basal controls; $^{@}P<0.05$ vs. sham-operated controls; $^{\#}P<0.05$ vs. OVX controls; $^{*}P<0.05$ vs. OVX + 1 mg.

increases in weight of the liver (42%), lungs (19%), spleen (14%) and adrenal glands (39%) were seen when compared to those of sham controls.

Effects in tibial length

Figure 3 shows the changes in left tibial length: 37.7 ± 0.92 mm at day 0 (3 months of age) and 39.0 ± 0.4 mm at day 90 (6 months of age), a significant increase with age. The tibial length in OVX rats treated with 1 and 6 mg PGE_2/kg/day were longer compared to sham-OVX (39.7 ± 0.56 mm at 1 mg and 39.4 ± 0.31 mm at 6 mg vs. 39.0 ± 0.4 mm at sham-OVX).

Qualitative observation of microradiographs

The primary spongiosa and growth plate were thinner in sham controls than in basal controls (Figs. 4B vs. A). No obvious bone mass change was found in the secondary spongiosa between basal (3 months of age) and sham (6 months of age) controls. Thinner and more dense primary spongiosa and less bone mass

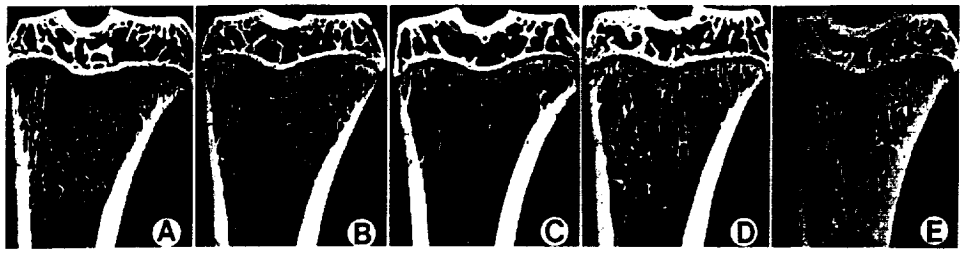

Fig. 4. Microradiographs showing cancellous bone changes in proximal tibial metaphyses from basal (A), sham-operated (B), OVX (C) controls and OVX rats treated with 1 (D) and 6 (E) mg PGE_2/kg/day. Thinner primary spongiosa and less bone mass throughout the whole metaphysis were observed in OVX control (C) than that in the sham control (B). Bone mass in OVX rat treated with 1 mg of PGE_2 (D) was greater than that in OVX control (C) and about equal to that in sham control (B). Bone mass in OVX rat treated with 6 mg of PGE_2 (E) was greater than that in both OVX (C) and sham (B) controls (× 10.5).

throughout the entire metaphysis were observed in OVX controls than in sham controls (Figs. 4C vs. B). In the 1-mg PGE_2/kg/day-treated OVX rat metaphysis, the primary spongiosa was wider, the bone mass was greater, and the trabeculae were thicker than in OVX controls (Figs. 4D vs. C), and equal to that in sham controls (Fig. 4B). The primary spongiosa width, bone mass and trabecular width in the 6-mg PGE_2/kg/day-treated OVX rat metaphysis (Fig. 4E) was much wider than that in any other group (Figs. 4A–D).

Effects on bone mass and microanatomical structure

Figures 5–7 and Table 1 show that trabecular bone area, width, number, separation and microanatomical structural indices changed in aging, OVX controls and OVX rats treated with 1 and 6 mg PGE_2/kg/day.

Effects of aging. Between 3 and 6 months of age, there was no significant difference in trabecular bone area, width, number and separation, and all tissue-

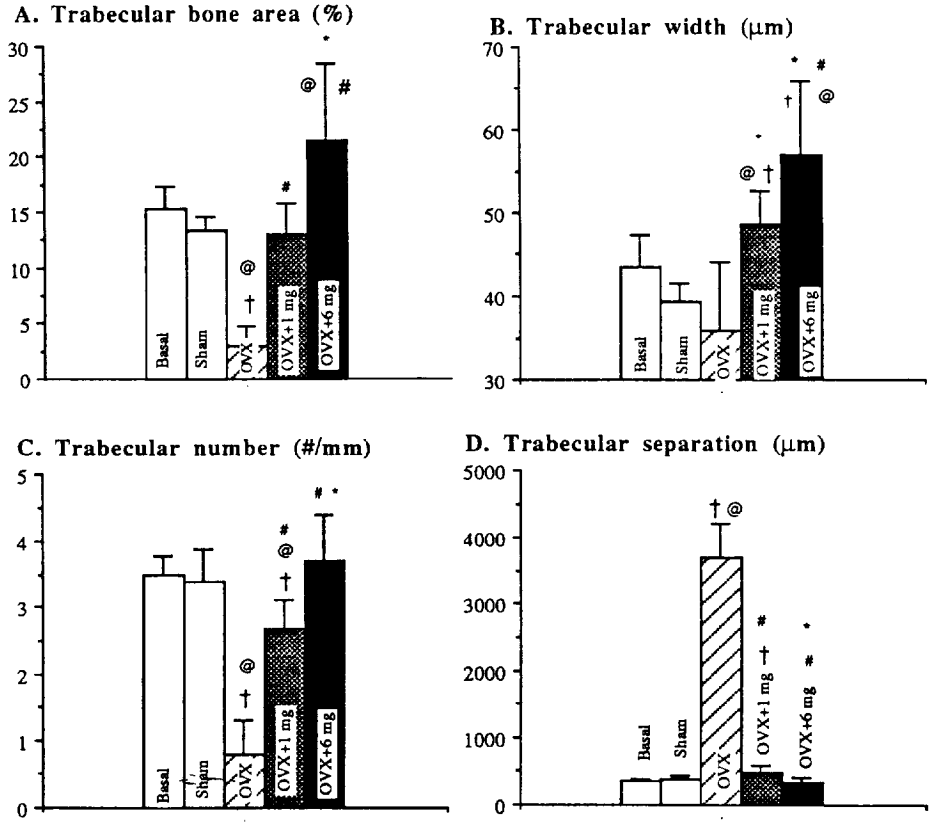

Fig. 5. Static histomorphometric indices of the proximal tibial metaphyseal trabeculae in basal, sham-operated and OVX controls and PGE_2-treated OVX rats. Y error bar represents standard deviation. $^{\dagger}P<0.05$ vs. basal controls; $^{@}P<0.05$ vs. sham-operated controls; $^{\#}P<0.05$ vs. OVX controls; $^{*}P<0.05$ vs. OVX + 1 mg.

based structural indices (Figs. 5 and 6, Table 1). However, when sham controls were compared to basal controls, the trabecular-bone-based density of node, node to node, and node to free end increased while cortex to free end and free end to free end decreased (Fig. 7).

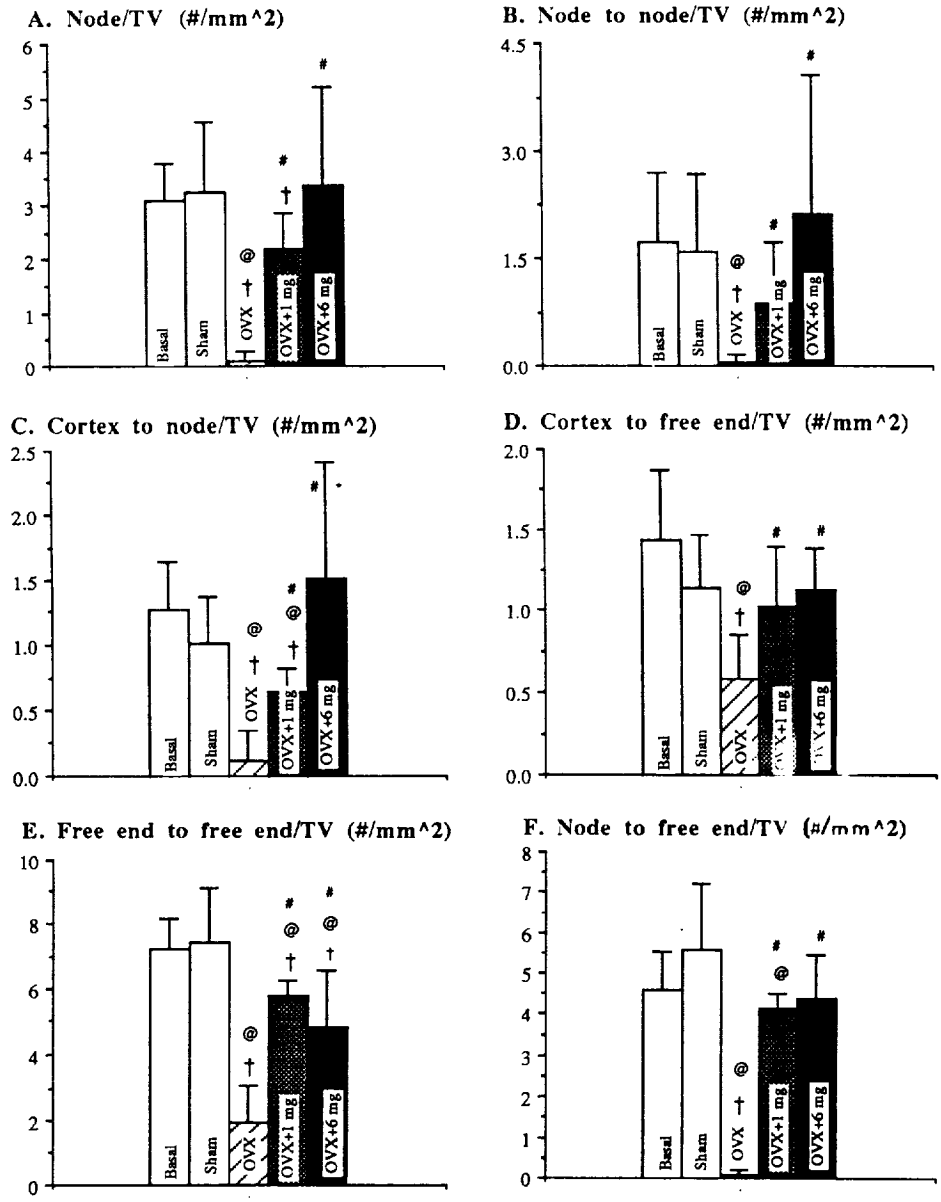

Fig. 6. Tissue-area-based microanatomical structural indices of the proximal tibial metaphyseal trabeculae in basal, sham-operated, OVX controls and PGE_2-treated OVX rats. Y error bar represents standard deviation. $^{\dagger}P<0.05$ vs. basal controls; $^{@}P<0.05$ vs. sham-operated controls; $^{\#}P<0.05$ vs. OVX controls; $^{*}P<0.05$ vs. OVX + 1 mg.

Effects of OVX. Compared to sham-operated controls, OVX resulted in: (1) an 80% decrease in percent trabecular bone area, and a 77% decrease in trabecular number; (2) 960% increase in trabecular separation (Fig. 5); (3) a decrease in all tissue-based microanatomical structural indices (from 60% to 97%, Fig. 6); (4) a

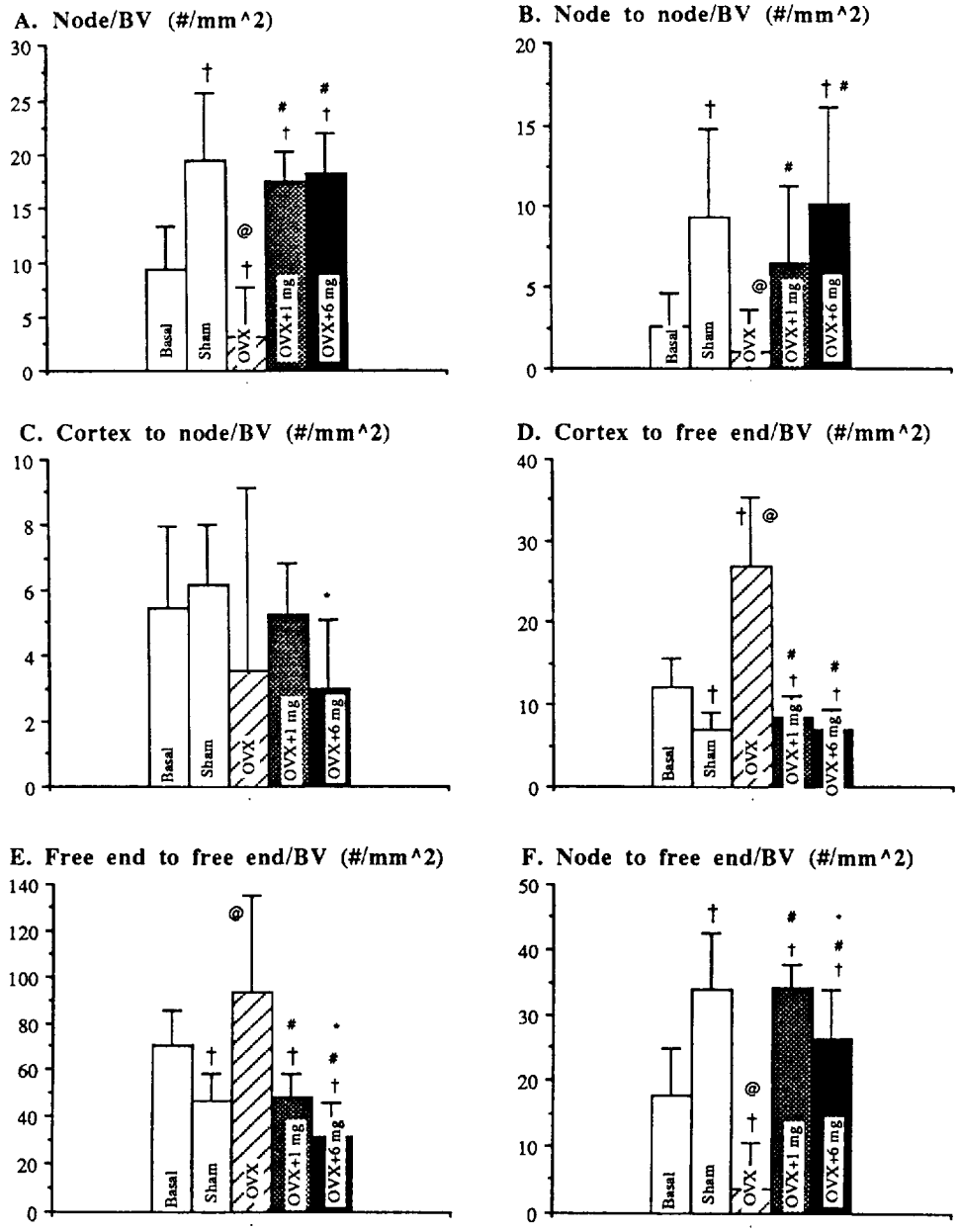

Fig. 7. Trabecular-bone-area-based microanatomical structural indices of proximal tibial metaphyseal trabeculae in basal, sham-operated, OVX controls and PGE_2-treated OVX rats. Y error bar represents standard deviation. $^{\dagger}P<0.05$ vs. basal controls; $^{@}P<0.05$ vs. sham-operated controls; $^{\#}<0.05$ vs. OVX controls; $^{*}P<0.05$ vs. OVX + 1 mg.

decrease in trabecular-bone-based density of node (85%), node to node (89%), node to free end (90%), and an increase in the indices for free end to free end (102%) and cortex to free end (282%) (Fig. 7). The ratio of node to free end decreased to 13% of those of basal and sham controls (Table 1).

Effects of PGE_2 on OVX rats (compared to OVX controls). In PGE_2-treated OVX rats, trabecular bone area, width and number increased and trabecular separation decreased. Trabecular bone area increased 343 and 626% over OVX controls for 1- and 6-mg PGE_2/kg/day-treated OVX rats. Similarly trabecular width increased 35% for the 6-mg dose, and trabecular number increased 233% and 359% for the 1- and 6-mg doses. Trabecular separation decreased 87% and 91% for 1- and 6-mg PGE_2/kg/day-treated OVX rats (Fig. 5). In 1- and 6-mg PGE_2/kg/day-treated OVX rats, all tissue-based structural indices increased from 79 to 2187% (Fig. 6). Trabecular-bone-based density of node, node to node and node to free end were elevated while free end to free end and cortex to free end declined in 1- and 6-mg PGE_2/kg/day-treated OVX rats (Fig. 7). Further, there were 6- and 10-fold increases in node to free end ratios after 1 and 6 mg PGE_2 treatment (Table 1).

Effects of PGE_2 on OVX rats (compared to sham controls). In PGE_2-treated OVX rats, trabecular bone area in 1-mg PGE_2/kg/day-treated OVX rats was unchanged (Fig. 5A), but trabecular number declined by 21% and trabecular width was elevated by 23% (Figs. 5B and C). At the 6-mg dose level, percent trabecular bone area increased by 61% and trabecular width by 44% (Fig. 5). In 1-mg PGE_2/kg/day-treated OVX rats, the tissue-based density of cortex to node, free end to free end and node to free end were lower than sham controls (22 to 37%) (Fig. 6). None of the trabecular bone-based microanatomical structure indices differed from sham controls at both 1- and 6-mg dose levels (Fig. 7).

Effects on longitudinal bone growth and growth plate thickness

When compared to the rate of longitudinal growth at 3 months of age, the longitudinal growth rate at 6 months of age had decreased by 71%. However OVX controls showed a 21% increase in longitudinal growth rate over sham-

Table 1

Ratio of node to free end of proximal tibial trabeculae[a]

	Ratio	vs. basal	vs. sham	vs. OVX	vs. OVX + 1 mg
Basal controls	0.23 ± 0.04				
Sham controls	0.23 ± 0.08	−3% (ns)			
OVX controls	0.03 ± 0.05	−87% ($P<0.001$)	−87% ($P<0.001$)		
OVX + 1 mg	0.20 ± 0.05	−15% (ns)	−13% (ns)	+578% ($P<0.05$)	
OVX + 6 mg	0.34 ± 0.22	+45% (ns)	+50% (ns)	+1054% ($P<0.05$)	+72% (ns)

[a] Calculated as: node no./(free end to free end no. + cortex to free end no. + node to free end no.).
ns: nonsignificant difference.

OVX controls (Fig. 8A). In the 6-mg PGE_2-treated OVX rats, longitudinal growth rate increased compared to sham (48%) and OVX (22%) controls (Fig. 8A). Growth plate thickness decreased in sham controls compared to basal controls, while it increased in ovariectomized rats compared to sham controls (33%). During the experimental periods, there was a 5.8 ± 0.1-mm new metaphysis generated (mean accumulated longitudinal growth) in sham-OVX control rats, while there was a metaphysis of 6.1 ± 0.3, 6.3 ± 0.2 and 6.5 ± 0.2 mm in OVX controls, 1- and 6-mg PGE_2-treated OVX rats, respectively (Fig. 8C); an increase in the latter three groups from the sham controls (Fig. 8C).

Effects on dynamic histomorphometry

Effects of aging. There were significant decreases in labeled perimeter (33%), bone- and tissue-area-based bone formation rates (38 and 46%, respectively), and significant increases in the formation (49%) and resorption (112%) periods in sham-OVX controls (6 months of age) compared to basal controls (3 months of age) (Figs. 9B, D and E, 10B and C).

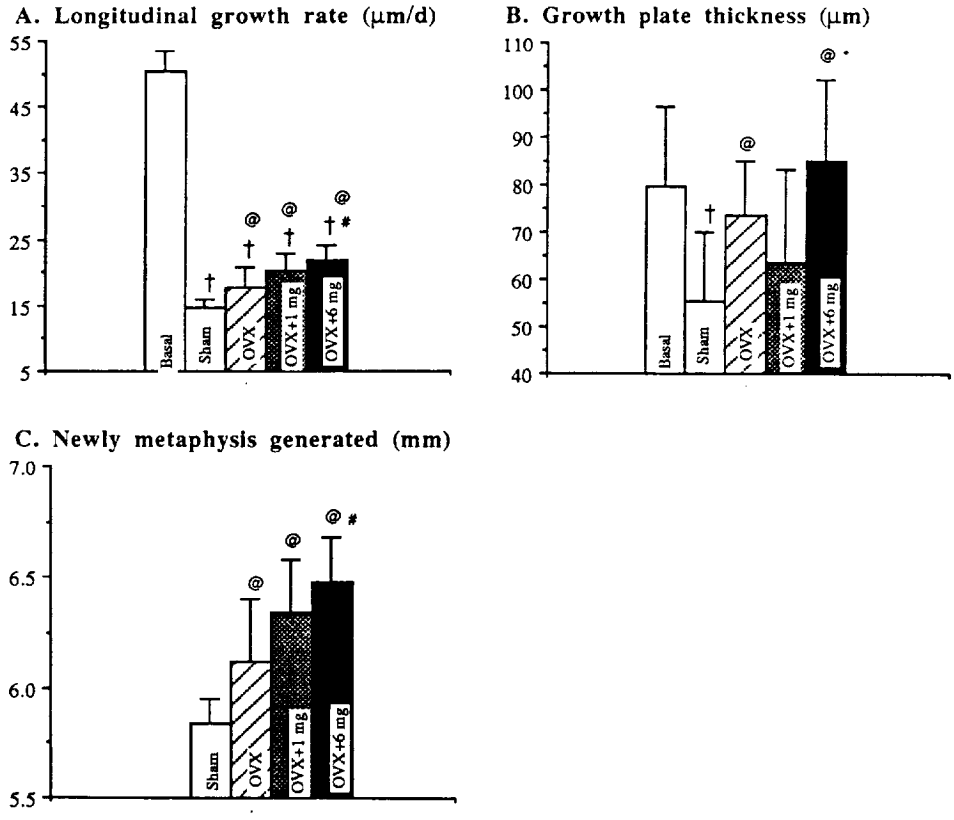

Fig. 8. Longitudinal growth rate, growth plate thickness and newly generated metaphysis of proximal tibial metaphyses in basal, sham-operated, OVX controls and PGE_2-treated OVX rats. Y error bar represents standard deviation. $^{\dagger}P<0.05$ vs. basal controls; $^{@}P<0.05$ vs. sham-operated controls; $^{\#}P<0.05$ vs. OVX controls; $^{\bullet}P<0.05$ vs. OVX + 1 mg.

Effects of ovariectomy. There were increases in percent osteoid perimeter (167%), labeled perimeter (109%), bone formation rate-bone area referent (126%), eroded perimeter (80%) and activation frequency (245%), and

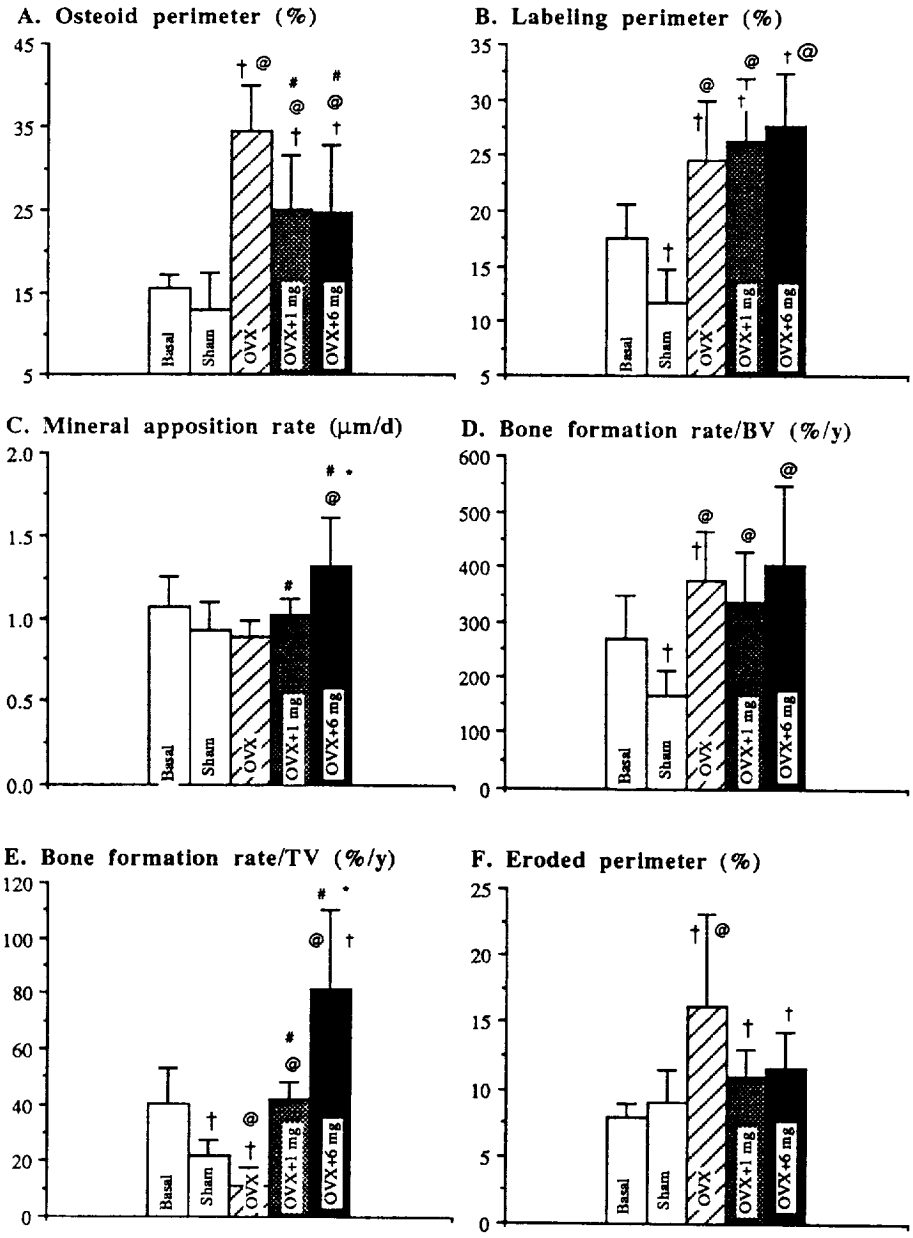

Fig. 9. Dynamic histomorphometric indices of the proximal tibial metaphyseal trabeculae in basal, sham-operated, OVX controls and PGE_2-treated OVX rats. Y error bar represents standard deviation. $^{\dagger}P<0.05$ vs. basal controls; $^{@}P<0.05$ vs. sham-operated controls; $^{\#}P<0.05$ vs. OVX controls; $^{*}P<0.05$ vs. OVX + 1 mg.

significant decreases in bone formation rate-tissue referent (51%), trabecular wall width (42%), formation period (39%), resorption period (70%), quiescent period (83%) and remodeling period (52%) in OVX controls compared to sham-OVX controls (Figs. 9 and 10).

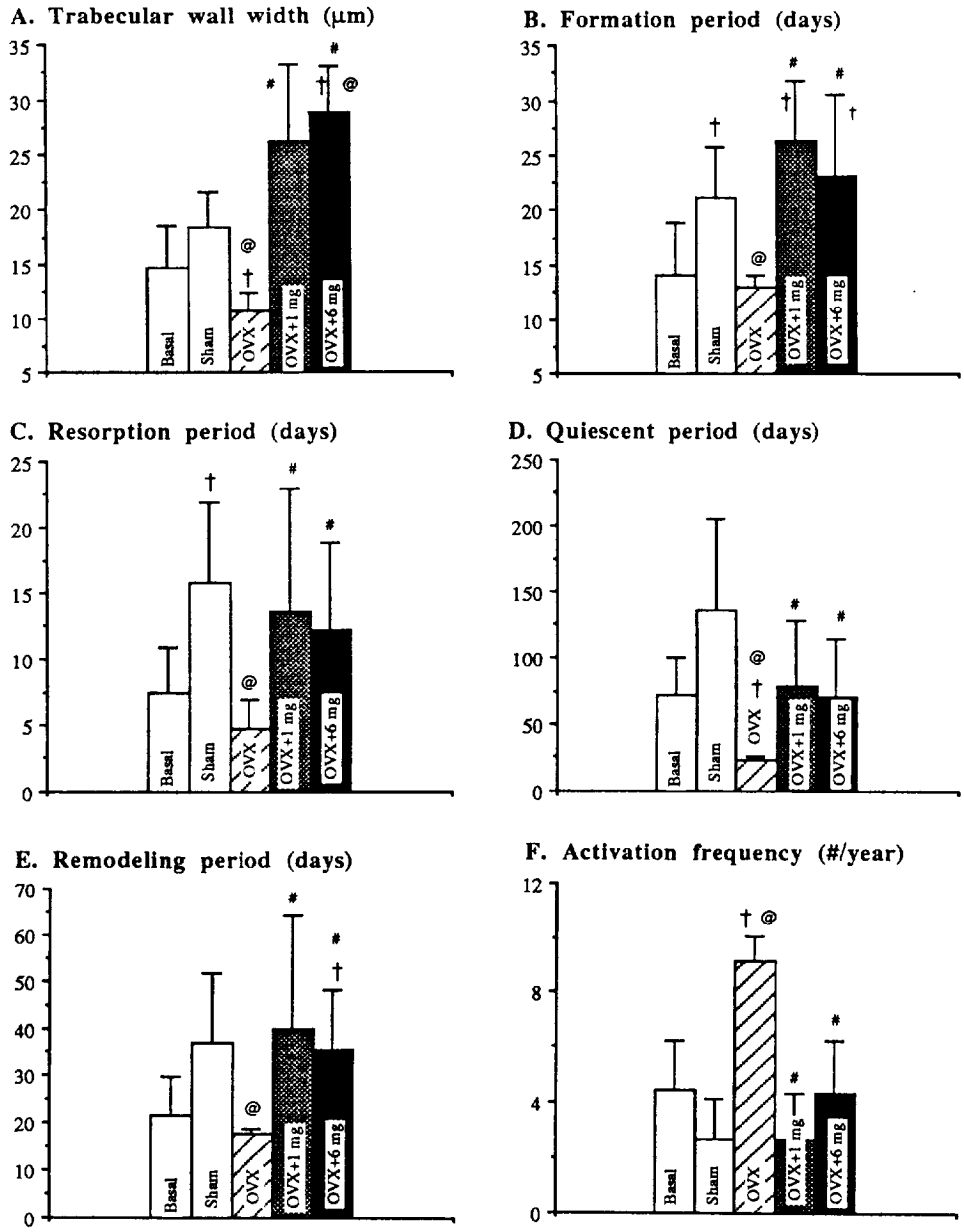

Fig. 10. Trabecular wall width, formation, resorption and quiescent periods and activation frequency of proximal tibial metaphyseal trabeculae in basal, sham-operated, OVX controls and PGE_2-treated OVX rats. Y error bar represents standard deviation. $^{\dagger}P<0.05$ vs. basal controls; $^{@}P<0.05$ vs. sham-operated controls; $^{\#}P<0.05$ vs. OVX controls; $^{*}P<0.05$ vs. OVX + 1 mg.

Effects of PGE_2 on OVX rats (compared to OVX controls). There was a significant decrease in percent osteoid perimeter (27 and 28%), and a significant increase in mineral apposition rate (15 and 29%), bone formation rate–tissue area referent (291 and 652%) and wall width (146 and 170%) in 1- and 6-mg PGE_2-treated OVX rats (Figs. 9 and 10A). Bone formation, resorption, quiescent and remodeling periods were shortened (from 79 to 244%), and the index of activation frequency of bone remodeling decreased (71 and 53%) in PGE_2-treated OVX rats (Figs. 10B–F).

Effects of PGE_2 on OVX rats (compared to sham-OVX controls). Except for mineral apposition rate in 1-mg PGE_2-treated OVX rats, all bone formation parameters were either increased significantly (42 to 270%) or, in the case of the bone resorption parameter, was not changed in the 1- and 6-mg PGE_2/kg/day-treated OVX rats (Figs. 9A–F). Wall width was thicker by 56% in 6-mg PGE_2-treated OVX rats (Fig. 10A). Bone formation, resorption, quiescent and remodeling periods, and activation frequency were nonsignificantly changed in PGE_2-treated OVX rats (Figs. 10B–F). Furthermore, diffuse-labeled new bone, as an index of woven bone formation, was seen in all 1- and 6-mg PGE_2/kg/day treated rats (0.011 ± 0.006 mm^2 at 1 mg and 0.022 ± 0.004 mm^2 at 6 mg, respectively) and was not seen in any basal, sham-OVX or OVX controls.

Discussion

Some interesting cancellous bone data were generated between sham-operated controls (6 months of age) and basal controls (3 months of age). Between 3 and 6 months of age, the trabecular bone area, width and number was nonsignificantly decreased in proximal tibial metaphyses. But the dynamic parameters show that trabecular labeling perimeter, bone formation rates (bone area and tissue area referent) and longitudinal growth rates declined significantly, while formation and resorption periods were elevated significantly, and trabecular eroded perimeter was not significantly different. Taken all together, these changes indicate that aging decreases bone formation, prolongs bone remodeling, but does not change bone resorption. The age-related changes in the indices of trabecular microanatomical structure indicate that the nonsignificant decrease in trabecular bone area between these ages coincides with the thinning of trabeculae and the loss of some disconnected trabeculae (CTF and FTF). At the same time there was no loss of connected trabeculae (NTN). The net effect was that the connections per unit bone area (node/BV and NTN/BV) were improved. Possibly, the explanation is that the mechanical loading on disconnected trabeculae is less than that in connected trabeculae, so that the disconnected trabeculae will be the first to go in age-related bone loss.

The ovariectomy-induced bone loss rat model is widely accepted for studying the prevention and treatment of postmenopausal osteoporosis [39–40]. In younger rats, ovariectomy results in a dramatic decrease in cancellous bone mass

associated with an increase in bone turnover with bone resorption exceeding formation [38–46]. Our findings of a decrease in trabecular bone area, the increment of percent eroded perimeter, percent osteoid and labeling perimeter, bone formation rate–bone area referent, and the decrement of formation, resorption, remodeling and quiescent periods in OVX control rats are consistent with findings reported previously [38–46]. Mineral apposition rate was increased significantly in OVX rats in other reports [23,44,47], but this change was not seen in the current study. Wronski et al. reported that longitudinal growth in proximal tibial metaphyses increased significantly in OVX rats 14 and 35 days post ovariectomy and returned to control levels thereafter [23,38,42–44]. However, we still observed a significantly increased longitudinal growth rate at 90 days post ovariectomy. The activation frequency index was elevated in our OVX rats which further confirms the increment in bone turnover. Even though bone formation per unit bone area increased in OVX rats, bone formation rate–tissue area referent declined in OVX rats, indicating the absolute bone-forming area decreased in these rats.

We also detailed the changes in microanatomical structural indices of trabecular bone in proximal tibial metaphyses in OVX rats in the current paper, data lacking in previous reports [38–46]. These indices showed that the OVX-induced bone loss is accompanied by decreased interconnectedness of normal trabecular structural pattern. The decreased interconnectedness of trabeculae was most obvious when one compares the ratio of node to free end between OVX and basal and sham-OVX control rats (Table 1).

The findings from the present study demonstrate that bone loss due to ovarian hormone deficiency can be prevented by a low-dose (1 mg) daily administration of PGE_2. Furthermore a higher-dose (6 mg) daily administration of PGE_2 not only prevents bone loss but also adds extra bone to the proximal tibial metaphyses. PGE_2 at the 1-mg dose level significantly increased trabecular bone area, trabecular width, trabecular node density, density of node to node, ratio of node to free end, and thus significantly decreased trabecular separation from OVX controls. At this dose level, these same parameters did not differ significantly from sham-operated controls. However, at the 6-mg dose level PGE_2, there were significant increases in trabecular bone area, trabecular width, trabecular node density, density of node to node, ratio of node to free end, while there was a significant decrease in trabecular separation from both OVX and sham-operated controls. The changes in indices of trabecular bone microanatomical structure indicated that PGE_2 prevented bone loss as well as the disconnection of existing trabeculae (Table 1).

Prevention of bone loss after ovariectomy by many agents, such as estrogen, parathyroid hormone, bisphosphonate have been studied [39,40,47–58]. Estrogen prevents bone loss induced by ovariectomy by inhibiting bone turnover and bone resorption [39,40,47–50]. Bone loss after ovariectomy can be prevented and restored by human parathyroid hormone by retarding bone resorption and enhancing bone formation [52–57]. Bisphosphonate decreases both bone formation and resorption in ovariectomized rats so as to prevent bone loss [58]. Unlike

the other agents [39,40,47–58], PGE_2 given to OVX rats does not inhibit the increment in percent eroded perimeter induced by ovariectomy, instead it stimulates lamellar bone formation, activates woven bone formation and creates a positive bone balance. Surprisingly, PGE_2-treated OVX rats had lower bone turnover rate than the OVX controls did (decrease in activation frequency index, and increase in formation, resorption and quiescent periods), since we previously reported that when PGE_2 was given to intact rats for 30 and 60 days, it increased bone turnover in favor of bone formation compared to age-related controls [16–22].

Finally, we conclude that PGE_2 can prevent bone loss due to estrogen deficiency (ovariectomy) and add extra cancellous bone to these ovariectomized rats by stimulating bone formation exceeding resorption, and activating woven bone formation. Our findings support the strategy of the use of bone stimulation agents in place of anti-resorptive agents in the prevention of estrogen depletion bone loss (postmenopausal osteoporosis).

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