Table 6. Correlation coefficient (R) for urinary deoxypyridinoline at 3 and 9 months in OVX rats treated with risedronate or vitamin K_2

Lumbar vertebra	Group	BMD	BV/TV	Ultimate load	Structural modulus	Mineral/matrix ratio	Carbonate/phosphate ratio
3 months	OVX-treated group (groups 2–7) (n = 59)	−0.640**	−0.696**	−0.544**	−0.542**	−0.340**	−0.121
	OVX-risedronate treated groups (groups 2–5) (n = 39)	−0.673**	−0.728**	−0.572**	−0.537**	−0.329*	−0.035
9 months	OVX-treated group (groups 2–7) (n = 59)	−0.685**	−0.722**	−0.542**	−0.553**	−0.425**	−0.196
	OVX-risedronate treated groups (groups 2–5) (n = 39)	−0.744**	−0.780**	−0.592**	−0.605**	−0.422**	−0.103

Femur	Group	BMD	Cortical area	Ultimate load	Structural modulus	Mineral/matrix ratio	Carbonate/phosphate ratio
3 months	OVX-treated group (groups 2–7) (n = 59)	−0.546**	−0.268*	−0.478**	−0.147	0.195	−0.032
	OVX-risedronate treated groups (groups 2–5) (n = 39)	−0.646**	−0.320*	−0.570**	−0.250	0.313	0.024
9 months	OVX-treated group (groups 2–7) (n = 59)	−0.571**	−0.257*	−0.476**	−0.141	0.126	0.044
	OVX-risedronate treated groups (groups 2–5) (n = 39)	−0.708**	−0.371*	−0.654**	−0.252	0.245	0.079

* $P < 0.05$, ** $P < 0.01$

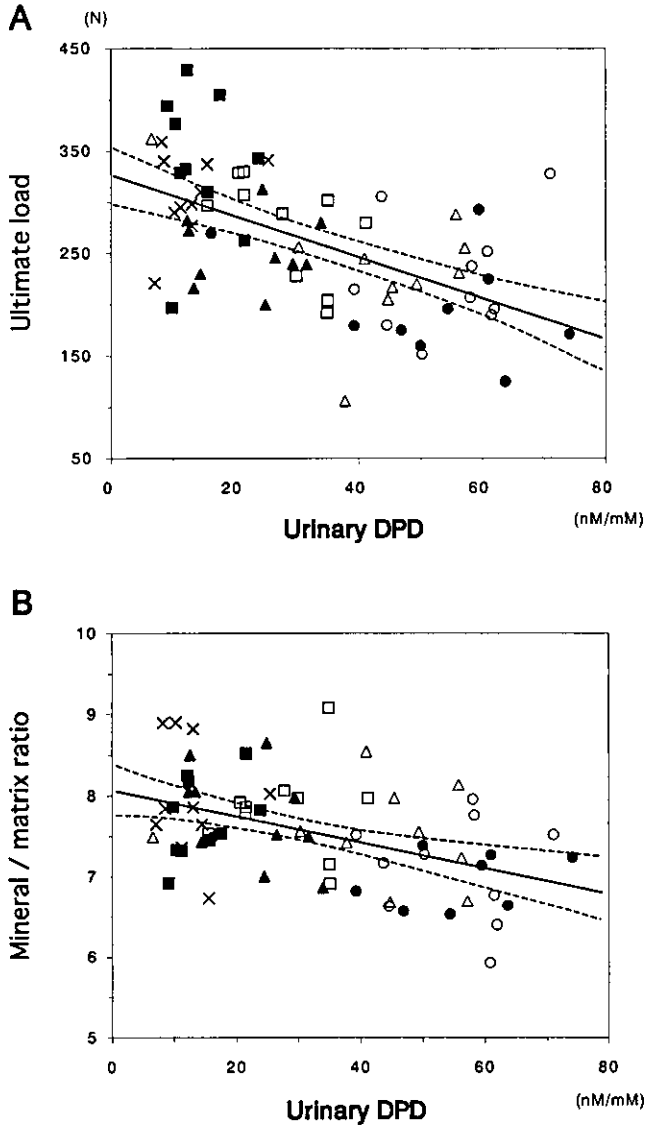

Fig. 2. Correlation between urinary deoxypyridinoline at 9 months after OVX and ultimate load (**A**) and mineral-to-matrix ratio (**B**) for the lumbar vertebra in OVX rats treated with risedronate or vitamin K_2. **A** Ultimate load = 327.95 − 2.01*urinary DPD, r^2 = 0.317, r = −0.563, $P < 0.0001$. **B** Mineral-to-matrix ratio = 8.064 − 0.016*urinary DPD, r^2 = 0.206, r = −0.454, $P < 0.0001$. *Solid line* in each panel indicates linear regression of data points shown; *broken lines* indicate 95% confidence limits. ×, group 1 (sham + vehicle); ●, group 2 (OVX + vehicle); △, group 3 (OVX + risedronate 0.1 mg/kg/day); ▲, group 4 (OVX + risedronate 0.5 mg/kg/day); ■, group 5 (OVX + risedronate 2.5 mg/kg/day); ○, group 6 (OVX + vehicle + vitamin K_2 30 mg/kg/day); □, group 7 (OVX + risedronate 0.5 mg/kg/day + vitamin K_2 30 mg/kg/day)

not prevent OVX-induced changes in bone mass, mechanical strength, and mineralization. The effects of the combination of vitamin K_2 and 0.5 mg/kg/day risedronate were not different from the effects of 0.5 mg/kg/day risedronate alone. Thus, although vitamin K_2 may have some action on serum osteocalcin levels, it did not substantially affect other parameters of bone measured in our study. Reported data on the effects of pharmacological doses of vitamin K_2 in OVX rats are inconsistent. Positive actions on bone mineral and strength have been observed with a daily dose of more than 30 mg given in the feed for 2–6 months after OVX in Wistar and Fischer rats [23,24]. However, treatment with daily doses of 22–49 mg for 12–14 weeks after OVX in SD rats did not show any positive effects on bone mass, strength, and structure [49,50]. We did not observe any positive effect with a daily dose of approximately 30 mg given in the feed for 9 months. Thus, the pharmacological action of vitamin K_2 on bone after OVX may depend on the strain of rats, the dose, and the period of administration.

In conclusion, this study demonstrated dose-related preservation of bone structure and quality with risedronate treatment in OVX rats. Importantly, risedronate did not increase the values of bone mechanical and mineral properties above the level for sham control animals. The strong association of bone turnover markers with mechanical and mineral properties of the vertebra suggests that the effects, at least in trabecular bone, were the result of dose-related suppression of bone turnover. The observed effects on mineral properties were different from those reported with other compounds.

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Human parathyroid hormone (1-34) increases mass and structure of the cortical shell, with resultant increase in lumbar bone strength, in ovariectomized rats

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Abstract Estrogen deficiency causes reduction of bone mass and abnormal bone microarchitecture, consequently reducing bone strength. Human parathyroid hormone (hPTH) (1-34) increases bone mass and strength. To clarify the factors that determine the recovery of bone strength in the lumbar vertebrae of ovariectomized rats by intermittent hPTH administration, we analyzed the relationship between skeletal measurements and bone strength. Human PTH (1-34) administration resulted in recovery of cortical bone mineral content (BMC) and cortical bone area to sham the levels, but in resulted in a less pronounced recovery of trabecular BMC and no increase in the total cross-sectional area of the vertebral body. Of the three-dimensional (3D) trabecular bone parameters, hPTH (1-34) increased trabecular thickness (Tb.Th). The cortical shell area of L4, determined by histomorphometry, was also increased. In hPTH-treated rats, the only determinant of the compressive load of L5 was the cortical shell BMC, in the early recovery period (days 42–84). Our data suggest that increased cortical bone mass contributes more than trabecular bone mass and structure to the recovery of bone strength in response to hPTH therapy in the rat lumbar vertebral body after ovariectomy.

Key words DXA · pQCT · micro-CT · bone strength · cortical shell

Introduction

Osteoporosis is a disease characterized by reduced bone mass and deterioration of the bone microstructure, consequently leading to a reduction of bone strength and increased risk of bone fractures [1]. Human parathyroid hormone (hPTH) (1-34) has a marked anabolic effect in increasing bone mass and strength in several species [2,3]. Studies in humans have confirmed that hPTH increases bone mass [4,5]. Treatment with recombinant hPTH (1-34) (teriparatide) for a median of 19 months has been shown to reduce the risk of vertebral fracture by 65% in a large multinational study of women with osteoporosis [6]. Teriparatide treatment increased lumbar bone mineral density (BMD) by 12% over 14 months [7]. However, data on the microstructure of bone in humans treated with hPTH are insufficient, with the exception of results in iliac bone specimens, obtained from a limited number of hPTH-treated patients, that showed a significant increase in cortical width, with a modest increase in trabecular bone volume [8,9]. Thus, the relationship between the reduction in the risk of vertebral fracture and improvements of trabecular and cortical bone structure has not been clearly elucidated in patients with osteoporosis treated with hPTH (1-34).

In rats, hPTH administration increases both trabecular and cortical bone mass [10–13]. It has been documented that administration of hPTH (1-34) for more than 6 months increases lumbar BMD, trabecular bone volume, and bone strength in ovariectomized rats [12,14–17]. The cortex serves as a constraint on the internal porous trabecular structure in the vertebral bones of rats [18], as it does in humans [19,20], and the relative contribution of trabecular bone to the strength of the vertebra varies depending on the mass and structure [21,22]. Thus, the cortical shell is expected to play an important role in the load-bearing capacity of the vertebral body when the trabecular bone is weakened after ovariectomy (OVX) in rats [23]. However, the contribution of the cortical shell to the mechanical strength of the vertebral body has not been well explored [18], nor has the recovery of vertebral bone strength in response to hPTH (1-34) administration been assessed in relation to bone mass and the structure of trabecular bone and cortical shell in ovariectomized rats.

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The present study was designed to examine the effects of increased mass and structure of the cortical shell, induced by hPTH (1-34) treatment, on vertebral bone strength. For this purpose, we first established osteopenic rats by performing OVX, and then assigned the rats (including sham-operated rats [sham]) to groups for vehicle or hPTH (1-34) treatment. Using serially obtained lumbar vertebral body specimens, we then performed in vitro measurements, including BMD and bone mineral content (BMC), in all samples, by dual-energy X-ray absorptiometry (DXA). The mass and structure of the cortical shell and trabecular bone was determined by peripheral quantitative computed tomography (pQCT) and histomorphometry, and the three-dimensional (3D) structure of trabecular bone was determined by micro-CT (μ-CT) and mechanical testing, using a materials-testing machine. After confirming the recovery of ultimate load in samples from the hPTH (1-34)-treated groups to that of the sham level, we then analyzed the contribution of each parameter to the load values in sham, OVX-control, and hPTH (1-34)-treated rats.

Materials and methods

Experimental animals

Female Sprague-Dawley (SD) rats (n = 217; 8 weeks of age) were purchased from Charles River Japan (Hino, Japan), and acclimatized for 8 weeks prior to the start of the experiment. The rats had free access to tap water and to a commercial standard rat chow containing 1.11% calcium and 1.08 IU/g of vitamin D3 (Oriental Yeast, Tokyo, Japan). All rats were housed in metal cages (3 or 4 rats per cage) in an air-conditioned environment (room temperature, 23 ± 2°C; humidity, 55 ± 10%) illuminated from 7:00 to 19:00. To control weight gain after OVX, the amount of food administered was adjusted to 60 g/kg body weight (BW) per day during the experimental period. BW was measured weekly. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the University of Occupational and Environmental Health.

Experimental design

At 16 weeks of age, rats were randomized by BW into 21 groups. Ten rats were killed on day 0 (baseline control). The other 207 rats underwent OVX (n = 135) or sham surgery (sham; n = 72) by the dorsal approach, under ether anesthesia. After surgery, all rats were maintained without treatment for 6 weeks. At 6 weeks, one group each from the OVX and sham rats was killed to evaluate pretreatment bone loss. The remaining rats were injected once a day, for 3 days a week, subcutaneously with vehicle or with hPTH (1-34) at 20 μg/kg. The sham groups were injected with vehicle only. Synthetic hPTH (1-34) was provided by Asahi Kasei (Shizuoka, Japan). The hPTH (1-34) sample was prepared in a vehicle of acidified saline containing 0.1% bovine serum albumin. Ten rats each from the OVX-control and hPTH-treated groups were killed on days 42, 56, 70, 84, 98, 112, and 126 post-surgery, 48 h after the last administration of hPTH.

Samples

Urine samples were collected from each rat (over a period of 24 h before they were killed) and stored at −80°C until use. Cardiac blood samples, obtained at the time the animals were killed under ether anesthesia, were immediately centrifuged, and serum samples were stored at −80°C. After exsanguination, the fourth (L4) and the fifth lumbar vertebrae (L5) were dissected carefully, and immediately stored at −80°C until use. The effect of OVX was verified by involution of the uterus.

Bone metabolic markers

Serum osteocalcin concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) kit, employing rat osteocalcin antibody (Amersham Pharmacia Biotech, Tokyo). The values for results were expressed as nanograms per milliliter. The limit of sensitivity for the assay was 0.05 ng/ml. Urinary deoxypyridinoline (DPD) cross-link excretion levels were measured using an ELISA kit (Pyrilinks-D; Metra Biosystems, Mountain View, CA, USA), and the values for results were expressed as DPD/creatinine (Cr) ratios (nmol/l/mmol/l Cr).

Cross-sectional bone mass and structure of L5 by pQCT

After the removal of adherent tissues and the posterior elements and transverse processes, one cross-sectional scan was made at the midpoint of the longitudinal axis of the L5 specimen, using pQCT (XCT Research; Stratec Medizintechnik, Pforzheim, Germany). This system has a 50-kV X-ray tube with a current of 0.288 mA as the source of a 0.5-mm-wide beam. A scout scan of the vertebra was performed, and, on the scout view, a reference line was manually placed such that the cross-sectional slice passed through the midpoint of the longitudinal axis of the vertebral body. The voxel size was 0.120 × 0.120 × 0.5 mm. When the vertebra was analyzed, the threshold for cortical bone was 690.0 mg/

cm^3 with a separation mode of 2, and the threshold for cancellous bone was 169.0 mg/cm^3, with a peel mode of 20 and a contour mode of 2. Upon completion of the scanning, the following parameters were analyzed for each slice, using the XCT 540 software. The values of the following parameters were then obtained: total cross-sectional BMC (total BMC; mg/mm), BMC of the cortical shell (cortical BMC; mg/mm), and BMD of the cortical shell (cortical BMD; mg/mm^3), trabecular BMC, total cross-sectional area (total Cr.Ar.; mm^2), and cortical shell bone area (Ct.B.Ar.; mm^2), as well as the periosteal circumference perimeter. The coefficient of variation (CV) values for total BMC, cortical BMC, cortical BMD, trabecular BMC, total Cr.Ar., and Ct.B.Ar. were 2.4%, 4.0%, 0.8%, 6.2%, 2.1%, and 4.8%, respectively.

3D-trabecular bone structure of L5 by μ-CT

Each L5 specimen was then scanned by μ-CT (μCT20; Scanco Medical, Zurich, Switzerland), as reported previously [20]. The whole L5 body was scanned dorsoventrally, using 200 slices with 8-μm slice thickness. To obtain the original 3D image of the L-5 body, a threshold value of 180 was used to binarize the spongiosa and cortex in this analysis system. This threshold value was selected on the basis of our previous study, in comparison to histological evaluation [24]. On 3D analysis, the tissue volume (TV; mm^3) and trabecular bone volume (BV; mm^3) were measured directly, and the fractional trabecular bone volume (trabecular BV/TV; %) was calculated. Trabecular thickness (Tb.Th; μm), trabecular separation (Tb.Sp; μm), and trabecular number (Tb.N; 1/mm) were measured directly on 3D images. For nonmetric indices, the values of 3D structural indices, including structure model index (SMI) and trabecular bone pattern factor (TBPf), were calculated using software included in the 3D μ-CT system. SMI represented the plate-rod characteristic of trabecular structure. TBPf represented the contour of the trabecular surface, i.e., the ratio of convex-to-concave surfaces in 2D cross-sectional images of trabecular bone. The CV values of trabecular BV/TV, Tb.Th, Tb.Sp, Tb.N, SMI, and TBPf were 3.47%, 2.69%, 1.02%, 1.12%, 12.6%, and 9.36%, respectively.

Bone size and bone mineral measurements by DXA

The height of the L5 body specimen was measured with a micrometer. The specimen was fixed with a clamp at the base of the transverse process in the holder of a diamond band saw (Exakt, Norderstedt, Germany). The plano-parallel surfaces were obtained by removing both the cranial and caudal endplates. From each vertebral body, a central cylinder specimen was obtained, with plano-parallel ends, measuring approximately 4.0 mm in height [12,23]. The volume of the specimen was measured by the Archimedes method, using an electric volumetric apparatus (MK-550; Muromachi Machinery, Tokyo). BMC (mg) and BMD (mg/cm^2) were measured for each specimen by DXA (DCS-600; Aloka, Tokyo) using the small-animal scan mode with irradiation applied anteroposteriorly to the specimen. The CV values of BMC and BMD were 1.53% and 1.05%, respectively.

Mechanical test

L5 vertebral cylinder samples were then placed centrally on the smooth surface of a steel disk (10-cm diameter) attached to a materials-testing machine (Tensilon UTA-1T; Orientec, Tokyo). A compression force was applied in a craniocaudal direction by using a steel disk (1.8-cm diameter) at a nominal deformation rate of 2 mm/min [12]. A load-deformation curve was displayed on the monitoring recorder linked to the tester in each specimen. The ultimate load (N) was measured directly from the load-deformation curve.

Histomorphometry

The L4 body specimen was embedded in methylmethacrylate. From the middle portion of the specimen, a 7-μm-thick cross-cut section was obtained at the level of the caudal margins of the pedicles, using a microtome (Polycut; Reichert-Jung, Nussloch, Germany). Histomorphometry was performed with a semiautomatic image-analyzing system linked to a light microscope (Cosomozone 1S; Nikon, Tokyo, Japan). For each section, we measured the cortical area of the anterior cortex and trabecular bone area at the central part of the marrow in a 0.75 mm × 0.75-mm-square region, using NIH image analysis software. The parameters of total cross-sectional area (mm^2), periosteal circumference perimeter (mm), cortical thickness (mm), cortical bone area (mm^2), trabecular bone area (trabecular BV/TV; %), and trabecular thickness (Tb.Th; μm) were obtained according to the parallel plate model [25].

Statistical analysis

All data values were expressed as means ± standard error of the mean (SEM). Respective data sets for sham, OVX-control, and hPTH (1-34)-treated groups were first compared by two-factor factorial analysis of variance (ANOVA) for group and time. If analysis confirmed a significant difference between groups, then the difference between values at each time point was assessed by the Tukey-Kramer post-hoc test. The rela-

tionships between ultimate load and the respective parameters obtained by DXA, μ-CT, and pQCT were analyzed by simple regression. Stepwise regression analyses were then performed to identify the parameters that significantly influenced ultimate load values. Stepwise regression analysis was carried out to assess the independent variable effect of each parameter on the ultimate compressive load. A forward selection procedure was used with an F value of more than 4.0 for entry. A value of $P < 0.05$ was considered a significant difference.

Results

Mortality, body weight (BW), and bone sizes of rats

Six rats died during surgery and 3 rats died postoperatively. The remaining 208 rats remained healthy during the experimental period. Compared to the sham groups, the body weight in the OVX-control and hPTH-treated groups was significantly increased at 14 days post-surgery and thereafter. No intergroup difference was found between OVX-control and hPTH-treated groups (data not shown). The height and volume of L5 body specimens were not different between the groups (data not shown).

Serum osteocalcin and urinary DPD levels

Serum levels of osteocalcin in the sham groups decreased in an age-dependent manner (Fig. 1A). The values in OVX-control groups remained higher than those in the sham groups throughout the experimental period. Human PTH treatment significantly increased serum osteocalcin concentrations, relative to those in the sham and OVX-control groups, up to 84 days after surgery. Urinary DPD levels were significantly higher in OVX-control rats compared to sham rats from 56 days after surgery (Fig. 1B). The values in hPTH-treated rats at 126 days were lower than those in OVX-control rats.

BMC and BMD of L5 by DXA

BMC and BMD values of L5 were significantly lower in OVX-control rats compared with sham rats throughout the experimental period. However, the BMC and BMD values in hPTH-treated rats were significantly higher than those in OVX-controls and were equal to those in sham groups from 84 and 56 days after surgery, respectively (data not shown).

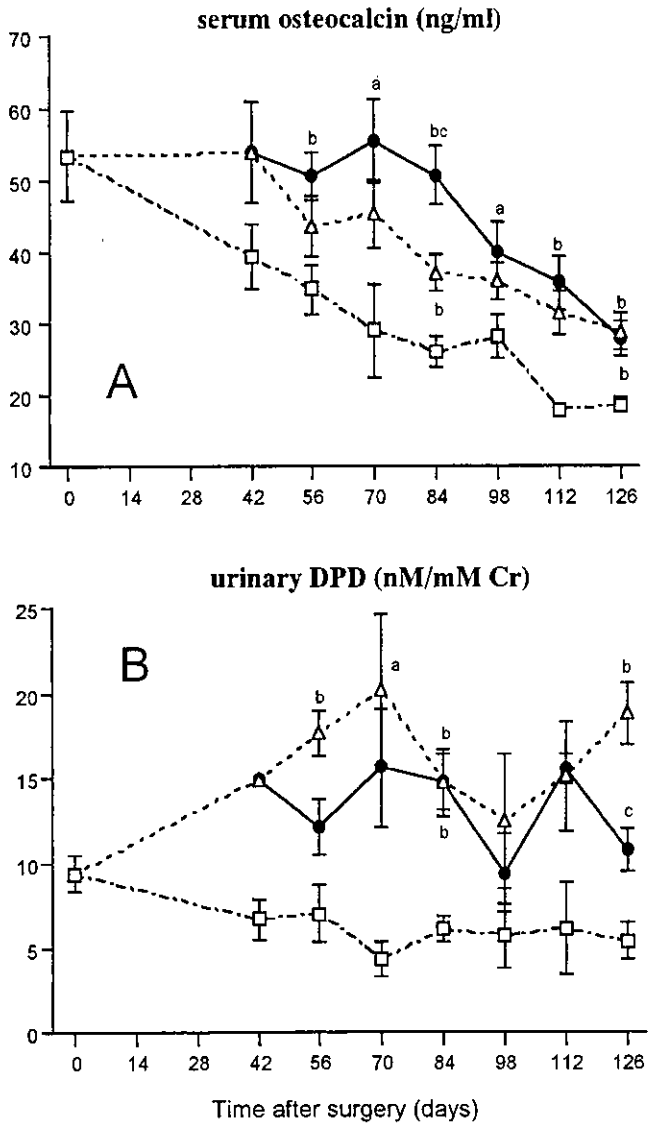

Fig. 1. Serial changes in the levels of bone metabolic markers. **A** Serum osteocalcin levels. **B** Urinary deoxypyridinoline (DPD) levels. Data values are expressed as means ± SEM. $^aP < 0.05$ and $^bP < 0.01$ vs sham group at the same time point. $^cP < 0.05$ vs OVX-control group at the same time point. *Open squares*, sham group; *open triangles*, ovariectomy (OVX)-control group; *closed circles*, human parathyroid hormone (hPTH)-treated group

Ultimate compressive load of L5 specimens

Compared to the sham groups, the ultimate load values of L5 cylinder specimens in the OVX-control groups were significantly decreased, by 15.6%, at 42 days, and were maintained at that level thereafter (Fig. 2). Treatment with hPTH resulted in significant increases in the load values, up to the level of sham groups at 84 days, and these effects were maintained at that level thereafter. Compared to the OVX-control groups, the compressive load in hPTH-treated rats was significantly larger at 70 days and thereafter.

Cross-sectional BMC, BMD, and structure by pQCT

Compared to the sham groups, the values for total cross-sectional BMC, cortical BMC, and trabecular BMC in the OVX-control groups were significantly lower at 42 days, but the values for cortical BMD were not different (Fig. 3A–D). Compared to OVX-controls, the parameters of total cross-sectional BMC and cortical BMC in the hPTH-treated groups were significantly higher from 70 days and were maintained at the level of the sham groups from 84 days. Cortical BMD, however, was significantly higher only at 126 days compared to that in OVX-controls. Treatment with hPTH also increased trabecular BMC from 70 days, although it did not reach the sham level. Cortical bone area, which was reduced in OVX controls from 42 days, similarly increased from 70 days. Total cross-sectional area was not significantly different among the three groups. Periosteal circumference perimeters were not different, either (data not shown).

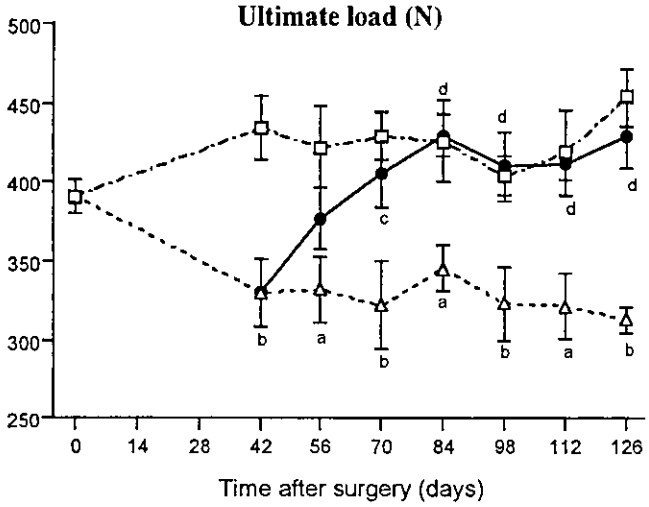

Fig. 2. Serial changes in ultimate compressive load of rat L5 vertebral body. Data values are expressed as means ± SEM. $^aP < 0.05$ and $^bP < 0.01$ vs sham group at the same time point; $^cP < 0.05$ and $^dP < 0.01$ vs OVX-control group at the same time point. *Symbols*, as in Fig. 1

3D trabecular bone structure by μ-CT

Treatment with hPTH resulted in an increase in the mean trabecular BV/TV from 70 days and thereafter, compared to the values in OVX-control groups, but the values remained significantly lower compared to those in sham groups (Fig. 4A). The Tb.Th values in sham groups increased with age, but the values remained stable at baseline levels in the OVX-control rats (Fig. 4B). Human PTH significantly increased Tb.Th

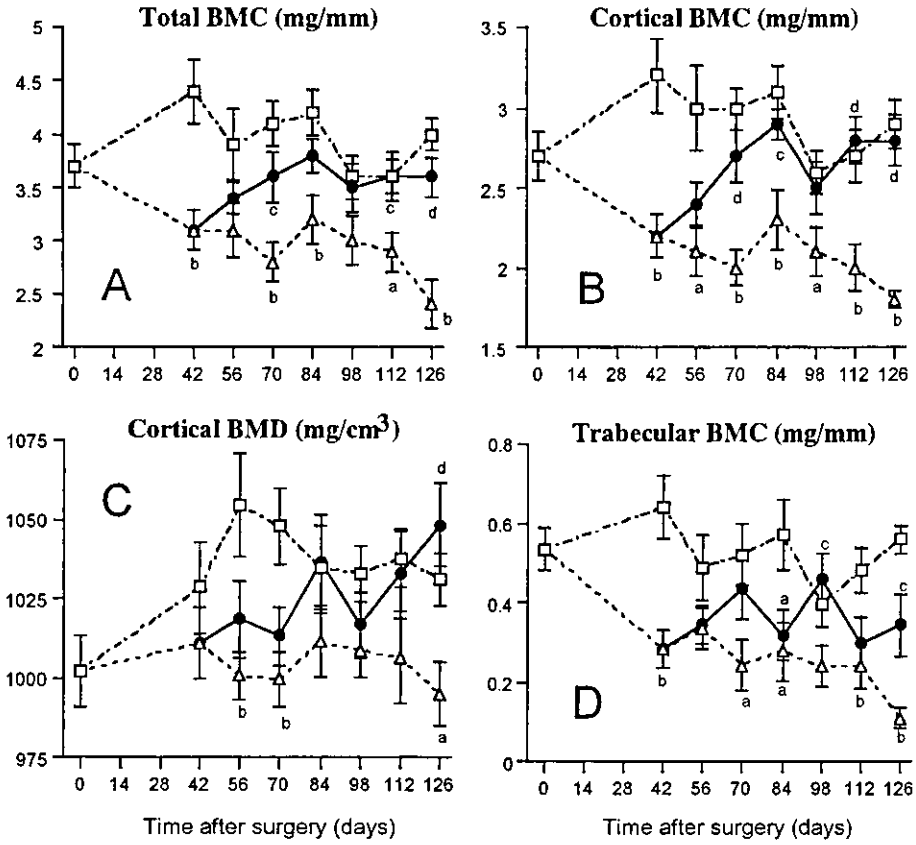

Fig. 3. Serial changes in total, cortical, and trabecular bone parameters of rat L5 vertebral body measured by peripheral quantitative computed tomography (pQCT). **A** Total cross-sectional bone mineral content (total BMC); **B** cortical shell bone mineral content (cortical BMC); **C** cortical shell bone mineral density (cortical BMD); and **D** trabecular bone mineral content (trabecular BMC) are shown. Data values are expressed as means ± SEM. $^aP < 0.05$ and $^bP < 0.01$ vs sham group at the same time point; $^cP < 0.05$ and $^dP < 0.01$ vs OVX-control group at the same time point. *Symbols*, as in Fig. 1

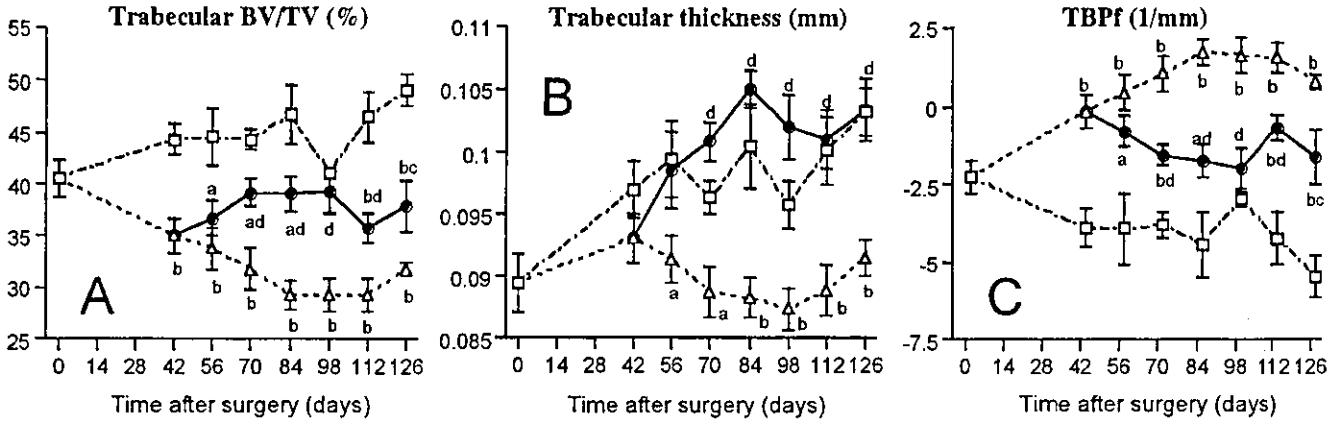

Fig. 4. Serial changes in three-dimensional and direct nonmetric microarchitectural indices of rat L5 vertebral body analyzed by micro-CT. **A** Trabecular bone volume (trabecular BV/TV); **B** trabecular thickness; and **C** trabecular bone pattern factor (TBPf) are shown. Data values are expressed as means ± SEM. $^{a}P < 0.05$ and $^{b}P < 0.01$ vs sham group at the same time point; $^{c}P < 0.05$ and $^{d}P < 0.01$ vs OVX-control group at the same time point. *Symbols*, as in Fig. 1

compared to that in OVX-controls from 70 days, while it did not improve Tb.N and Tb.Sp compared to that in OVX-controls and remained at significantly lower levels compared to those in sham rats (data not shown). The TBPf values in OVX-controls were significantly higher than those in sham rats from 42 days (Fig. 4C). Compared to the TBPf values in OVX-controls, hPTH significantly reduced these values from 70 days, but the values remained significantly higher than those of the sham groups. SMI values showed changes similar to those in TBPf (data not shown). The degree of anisotropy (DA) values in OVX-controls were significantly higher than those in sham rats from 42 days, and the DA values in hPTH-treated rats were significantly lower, from 56 days, than those in OVX-control rats (data not shown).

Histomorphometry

Compared to values in the sham groups, the values for L4 cross-sectional cortical bone area and cortical thickness in the OVX-control groups were significantly lower from 42 days (Fig. 5A,B; Fig. 6). Compared to the OVX-control groups, the parameters of cortical bone area and cortical thickness in the hPTH-treated groups were significantly higher from 56 days, and remained at the level of sham groups from 70 days. The values for trabecular bone volume (trabecular BV/TV; %) and the values for trabecular thickness in the OVX-control groups were significantly lower from 56 days (Fig. 5C,D) than those in the sham groups. Compared to OVX-controls, the parameters of trabecular BV/TV and Tb.Th in the hPTH-treated groups were significantly higher from 56 days. Total cross-sectional area and periosteal circumference perimeter values were not significantly different among the three groups (data not shown).

Simple regression analyses between the parameters of mass and structure and the ultimate load

The ultimate load values in hPTH-treated rats were apparently maintained from 84 days; therefore, the period from 42 to 84 days was defined as the early recovery period, while the period from 84 to 126 days was defined as the late maintenance period, in a post-hoc manner. Coefficient of determination values for the parameters obtained by DXA, pQCT, and μ-CT in sham, OVX-control, and hPTH-treated groups were calculated for the early recovery period and the late maintenance period (Table 1). In the sham groups, all the parameters of mass and structure significantly correlated with the ultimate load values. In the OVX-control rats, the values for total cross-sectional area by pQCT in the late period did not correlate with load values. In the hPTH-treated groups, the values of Tb.N and Tb.Sp in the early recovery period did not correlate with the ultimate load. The DA values in the OVX-control and the hPTH-treated groups did not correlate with load.

Stepwise regression analyses for the determinant factors of ultimate load

Among the 21 parameters of bone mass and structure that we examined, cortical BMC and TBPf in the early period and TBPf and BMC by DXA in the late period correlated significantly with the load values in the sham groups (Table 2). In the OVX-control groups, only the values for cortical bone area in the early period and the

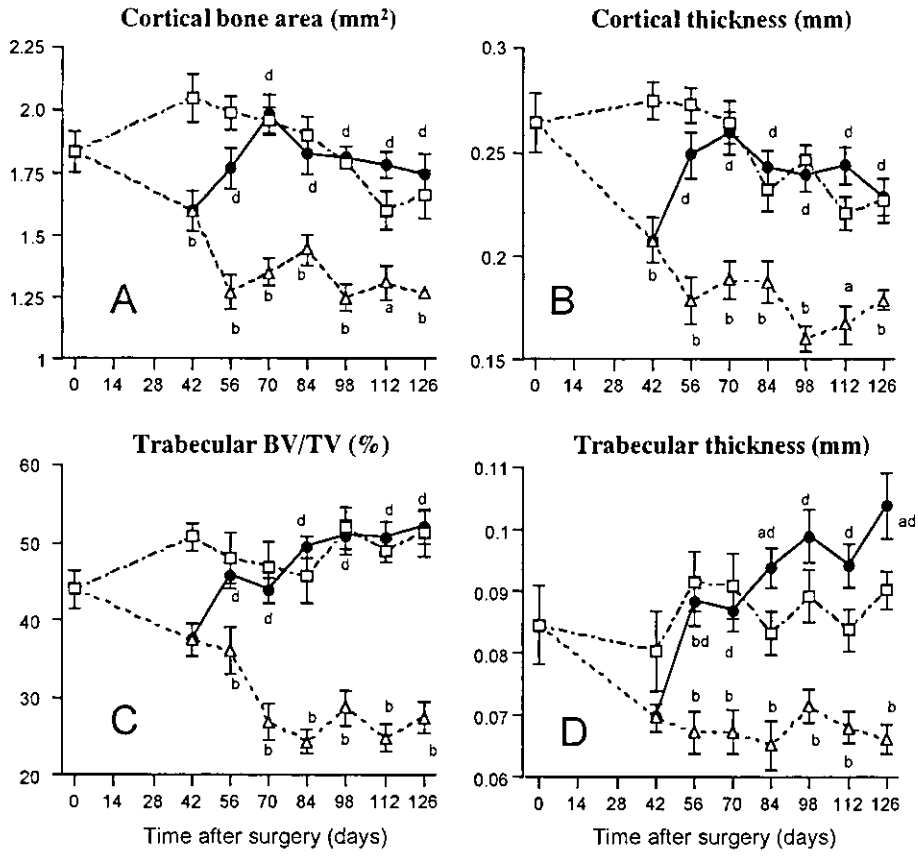

Fig. 5. Serial changes in cross-sectional morphometrical parameters of rat L4 vertebral body. **A** Cortical bone area; **B** cortical thickness; **C** trabecular bone volume (trabecular BV/TV; %); and **D** trabecular thickness are shown. Data values are expressed as means ± SEM. $^{a}P < 0.05$ and $^{b}P < 0.01$ vs sham group at the same time point; $^{d}P < 0.01$ vs OVX-control group at the same time point. *Symbols*, as in Fig. 1

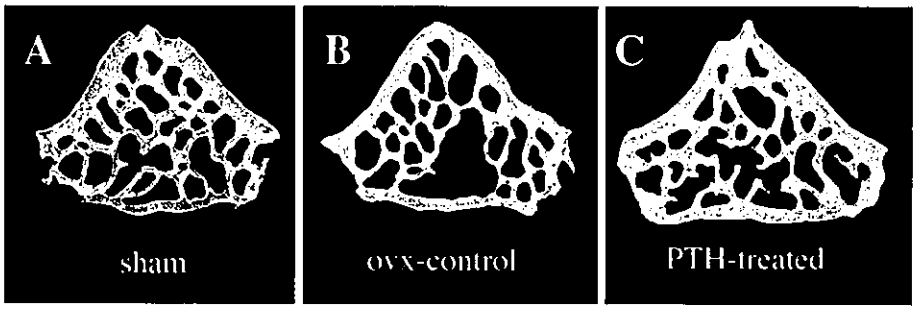

Fig. 6. Undecalcified middle cross-sections of L4 vertebral body at 126 days. **A** *Sham* groups; **B** *OVX*-control group, and **C** *hPTH* treated group. Cortical bone area, cortical thickness, and trabecular thickness were significantly increased in the hPTH-treated group, compared with values in the OVX-control group

values for cortical BMC and TBPf in the late period were significantly correlated with the load values. In the hPTH-treated groups, in the early recovery period, only cortical BMC was significantly correlated with the ultimate load values, with a standard regression coefficient value of 0.670. In the late maintenance period, the values for BMD and total cross-sectional BMC were significantly correlated with the load values, with a coefficient value of 0.828.

Discussion

This study demonstrated that hPTH (1-34) (20 μg/kg injected 3 days a week) restored BMC and the compressive strength of the lumbar vertebrae in rats with OVX-induced osteopenia, with increasing serum osteocalcin concentrations. Cross-sectional pQCT analyses of hPTH (1-34)-treated rats revealed that the cortical BMC and cortical bone area recovered to sham levels, although improvement of cortical BMD was delayed. Recovery of trabecular BMC was less pronounced, and there was no increase in the total cross-sectional area of the vertebral body. Of the 3D trabecular bone parameters, the Tb.Th values increased, but improvements in other parameters were less marked. In hPTH-treated rats, the only determinant of the compressive load of the lumbar bone was the cortical shell BMC, in the early recovery period, while the whole BMD was the dominant determinant in the late maintenance period. The

Table 1. Coefficient of determination (R^2) values for the ultimate load of L-5 between structural indices, bone mass, and bone metabolic markers analyzed by simple regression

	Sham		OVX		PTH	
	Early period (n = 36)	Late period (n = 33)	Early period (n = 39)	Late period (n = 37)	Early period (n = 40)	Late period (n = 37)
DXA (L5)						
BMC	0.682**	0.561**	0.481**	0.581**	0.485**	0.627**
BMD	0.684**	0.541**	0.413**	0.520**	0.469**	0.745**
μ-CT (L5)						
Trabecular BV/TV	0.594**	0.584**	0.261**	0.426**	0.371**	0.484**
Trabecular thickness	0.424**	0.531**	0.343**	0.249**	0.506**	0.435**
SMI	0.499**	0.565**	0.278**	0.317**	0.419**	0.454**
DA	0.417**	0.466**	0.009	0.048	0.063	0.093
Tb.Sp	0.496**	0.376**	0.153*	0.214**	0.004	0.217**
Tb.N	0.634**	0.528**	0.166*	0.307**	0.027	0.348**
TBPf	0.660**	0.669**	0.311**	0.463**	0.454**	0.507**
pQCT (L5)						
Total BMC	0.675**	0.569**	0.506**	0.320**	0.550**	0.688**
Total cross-sectional area	0.290**	0.191*	0.129*	0.027	0.108*	0.224**
Cortical BMC	0.704**	0.513**	0.566**	0.745**	0.666**	0.572**
Cortical BMD	0.012	0.026	0.027	0.063	0.041	0.004
Cortical bone area	0.697**	0.553**	0.579**	0.740**	0.656**	0.624**
Cortical thickness	0.621**	0.505**	0.434**	0.202**	0.402**	0.425**
Trabecular BMC	0.551**	0.601**	0.203**	0.228**	0.228**	0.230**
Histomorphometry (L4)						
Total cross-sectional area	0.120*	0.029	0.094	0.093	0.094*	0.111*
Cortical bone area	0.105	0.062	0.042	0.100	0.211**	0.158*
Cortical thickness	0.026	0.023	0.134*	0.122*	0.191**	0.045
Trabecular BV/TV	0.009	0.106	0.006	0.001	0.119*	0.092
Trabecular thickness	0.010	0.170*	0.005	0.005	0.112*	0.009

*$P < 0.05$; **$P < 0.01$ (simple regression)
The early period was between 42 and 84 days, and the late period was between 84 and 126 days postsurgery

Table 2. Determinants of ultimate compressive load analyzed by stepwise regression

	Early recovery period			Late maintenance period		
Group	Index	R^2	P value	Index	R^2	P value
Sham	Cortical BMC (pQCT)	0.743	<0.0001	TBPf (μ-CT)	0.669	<0.0001
	Cortical BMC (pQCT) + TBPf (μ-CT)	0.834	<0.0001	TBPf (μ-CT) + BMC (DXA)	0.746	<0.0001
OVX—control	Cortical bone area (pQCT)	0.554	<0.0001	Cortical BMC (pQCT)	0.774	<0.0001
				Cortical BMC (pQCT) + TBPf (μ-CT)	0.845	<0.0001
hPTH—treated	Cortical BMC (pQCT)	0.670	<0.0001	BMD (DXA)	0.766	<0.0001
				BMD (DXA) + Total BMC (pQCT)	0.828	<0.0001

The early recovery period was between 42 and 84 days, and the late maintenance period was between 84 and 126 days postsurgery

data for bone turnover, mass, structure, and strength of the vertebral body in the present study are consistent with those of previous studies in regard to the effects of hPTH injections at a frequency of three to seven times a week, at doses of 10–80 μg/kg, in mature [11,14,17,26–29], aged, ovariectomized rats [13,15,16,30,31], and ovariectomized cynomolgus monkeys [32]. Restoration of the mechanical strength of the vertebral body in osteopenic, ovariectomized rats is also compatible with the previous data [30]. Recombinant hPTH treatment, using daily doses of 5 μg/kg or more for up to 2 years in normal rats, led to profound skeletal changes, beyond normally attained peak levels, as evaluated by QCT [33]. The bone mass-increasing effects were markedly in excess of those observed in normal rats treated with similar doses of hPTH for up to 6 months [10,13,34]. In

humans, it has been observed that absolute changes in vertebral BMD after teriparatide therapy were independent of the initial BMD [35]. These data indicate that the effects of hPTH on bone mass and strength depend on the dose and duration of the treatment. However, the increases in mass and strength of the vertebral body in the present study did not exceed the levels in sham-operated rats. Thus, the dose of 20 μg/kg applied three times a week may not be potent enough to increase these parameters beyond the normal levels. Another explanation is that the lack of estrogen may reduce the capacity of hPTH treatment to augment the mass and strength of vertebral bone beyond the normal levels [36].

The absolute increase of cortical shell BMC in response to hPTH treatment was markedly greater than the increase of trabecular BMC. The recovery of cortical shell structure area was apparently complete, whereas parameters of trabecular structure did not reach the sham level, with the exception of Tb.Th. These data were in accordance with the previous observation that the effect of PTH injection was more pronounced in the cortical bone surfaces than in the trabecular bone surfaces in osteopenic, ovariectomized rats [12]. In contrast, these findings contradict a previous observation indicating a dominant effect of PTH on trabecular bone, determined by densitometry in humans [37], but are in accordance with the findings of a significant increase in cortical structure that was associated with only a modest increase in trabecular bone in iliac biopsy specimens from women with postmenopausal osteoporosis [8]. Periosteal expansion of the cortex in the long bones of the extremities has been confirmed in humans [38] and rats [12,25,39]. However, in our study, hPTH treatment did not change the total cross-sectional area of the lumbar bone, either as determined by pQCT or by histomorphometry and periosteal circumference perimeter (data not shown). Furthermore, the same treatment did not change the volume of mid-lumbar body specimens. These results indicate that, in conditions of estrogen deficiency, the periosteal surfaces of the axial skeleton are less sensitive to hPTH stimulation than those of the appendicular skeleton.

The concomitant increases in cortical shell BMC and area suggest that hPTH treatment increased the cortical shell BMC by increasing mineralized bone tissue. However, BMD was not increased substantially by the treatment. This discrepancy between the cortical shell BMC and BMD is consistent with previous data [39]. Because the BMD value obtained by pQCT represents the volumetric BMD, we can assume that the value reflects the mean degree of mineralization of the cortical shell at the tissue level. Thus, the delay in the increase in cortical shell BMD values relative to BMC may indicate that hPTH treatment did not substantially increase the mean degree of mineralization in the bone tissue. This finding is consistent with data from aged estrogen-depleted rats after long-term treatment with hPTH injections [16]. A marked heterogeneity of mineralization, including a higher percentage of less mineralized bone matrix, has been observed in iliac bone specimens obtained from women with postmenopausal osteoporosis after hPTH treatment [40]. Thus, it is expected that, in estrogen deficiency, the recovery of cortical bone mass and strength in response to hPTH is not associated with an increase in the mean degree of bone mineralization. Obviously, these data are in contrast to the concomitant increases of BMC and volumetric BMD of the cortical bone induced by bisphosphonates in estrogen deficiency [41].

When we compared the data of pQCT with those of histomorphometry, the value of cortical bone area, measured by histomorphometry, increased earlier than that measured by pQCT. When we compared the data of μ-CT with those of histomorphometry in trabecular bone, the values of trabecular BV/TV by histomorphometry had recovered substantially at 2 weeks of hPTH treatment. This finding is consistent with the data for iliac bone specimens from aged women after 1-year treatment with hPTH [42]. An explanation of this discrepancy may be that histomorphometry can detect poorly mineralized bone tissue, while radiological estimations, such as pQCT and μ-CT, detect sufficiently mineralized bone tissue.

Many parameters of bone mass and structure, including whole bone, cortical shell, and trabecular bone in the lumbar vertebra, correlated with the load values by simple regression analyses in sham, OVX-control, and hPTH-treated rats. Determinants of the lumbar bone strength included both mass and structure parameters of cortical shell and trabecular bone in both sham and OVX-control rats. Dominant parameters from the cortical shell and trabecular bone were not different between the early and late periods in either sham or OVX-control rats. Among the 3D-trabecular parameters, only the nonmetric parameters of TBPf and DA were significant determinants of lumbar bone strength. Thus, in the increasing of trabecular bone strength in the lumbar vertebra, the parameters of surface contour and the arrangement of trabecular bone structure, such as TBPf, seemed to be more responsible than the direct parameters of trabecular thickness and trabecular number, in both sham and ovariectomized rats, as previously reported [23]. In hPTH-treated rats, however, the only determinant of lumbar bone strength was the parameter of the cortical shell. Because the amount of mineralized bone tissue apposed in the cortical shell was larger than that in the trabecular bone, it is not unexpected that the cortical bone area was the dominant determinant of

bone strength during the early recovery period. In the late maintenance period, as the whole bone BMD by DXA was the most dominant determinant of strength, the composite structure of the cortical shell and trabecular bone appeared to have been reestablished after the recovery of strength in the lumbar bone.

Our study has several limitations. The modest dose, low frequency of injections, and relatively short period of experimentation may have led to an underestimation of the potency of hPTH to recover trabecular bone in the early period. The apparent unresponsiveness of the periosteal surface of the lumbar bone may also be due to a dosing effect. In addition, as trabecular bone is the dominant structure of the vertebral bone in humans, we must be careful in extending the biomechanical implications of lumbar bone findings in estrogen-deficient rats to those in women with postmenopausal osteoporosis. Despite all these limitations, the present findings provide important clues to our understanding of the mechanism underlying the potent anti-fracture effect of hPTH treatment in postmenopausal osteoporosis.

In conclusion, the present study demonstrated the dominant contribution of cortical shell structure to the recovery of vertebral bone strength in estrogen-deficient rats. An increase in the cortical shell BMC was associated with an increase in the mineralized bone tissue area. The anabolic effect of hPTH on the cortical component appears to be critical in preventing vertebral fractures, as it is in preventing nonvertebral fractures in postmenopausal osteoporosis.

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