JOURNAL OF BONE AND MINERAL RESEARCH
Volume 16, Number 1, 2001

Intermittently Administered Human Parathyroid Hormone(1-34) Treatment Increases Intracortical Bone Turnover and Porosity Without Reducing Bone Strength in the Humerus of Ovariectomized Cynomolgus Monkeys

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ABSTRACT

Cortical porosity in patients with hyperparathyroidism has raised the concern that intermittent parathyroid hormone (PTH) given to treat osteoporotic patients may weaken cortical bone by increasing its porosity. We hypothesized that treatment of ovariectomized (OVX) cynomolgus monkeys for up to 18 months with recombinant human PTH(1-34) [hPTH(1-34)] LY333334 would significantly increase porosity in the midshaft of the humerus but would not have a significant effect on the strength or stiffness of the humerus. We also hypothesized that withdrawal of PTH for 6 months after a 12-month treatment period would return porosity to control OVX values. OVX female cynomolgus monkeys were given once daily subcutaneous (sc) injections of recombinant hPTH(1-34) LY333334 at 1.0 μg/kg (PTH1), 5.0 μg/kg (PTH5), or 0.1 ml/kg per day of phosphate-buffered saline (OVX). Sham OVX animals (sham) were also given vehicle. After 12 months, PTH treatment was withdrawn from half of the monkeys in each treatment group (PTH1-W and PTH5-W), and they were treated for the remaining 6 months with vehicle. Double calcein labels were given before death at 18 months. After death, static and dynamic histomorphometric measurements were made intracortically and on periosteal and endocortical surfaces of sections from the middiaphysis of the left humerus. Bone mechanical properties were measured in the right humeral middiaphysis. PTH dose dependently increased intracortical porosity. However, the increased porosity did not have a significant detrimental effect on the mechanical properties of the bone. Most porosity was concentrated near the endocortical surface where its mechanical effect is small. In PTH5 monkeys, cortical area (Ct.Ar) and cortical thickness (Ct.Th) increased because of a significantly increased endocortical mineralizing surface. After withdrawal of treatment, porosity in PTH1-W animals declined to sham values, but porosity in PTH5-W animals remained significantly elevated compared with OVX and sham. We conclude that intermittently administered PTH(1-34) increases intracortical porosity in a dose-dependent manner but does not reduce the strength or stiffness of cortical bone. (J Bone Miner Res 2001;16:157–165)

Key words: parathyroid hormone(1-34), porosity, biomechanics, cortical bone, osteoporosis

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INTRODUCTION

HYPERPARATHYROIDISM HAS been associated with increased cortical porosity, which accompanies increased trabecular bone volume. Several small studies have shown that intermittently administered parathyroid hormone (PTH) given as therapy for osteoporosis also is associated with a loss of cortical bone from the peripheral skeleton,[1,2] although it increases cancellous bone volume in the vertebrae.[3,4] These phenomena raised concerns that the increases in vertebral bone volume and density stimulated by PTH are generated at the expense of cortical bone from the peripheral skeleton, the so-called cortical steal phenomenon, and might put cortical bone sites at increased risk of fracture. The cortical steal effect has been investigated in several different animal models with Haversian remodeling,[5–10] but small sample sizes and a lack of biomechanical data have precluded definitive results.

In a recent study using an ovary-intact rabbit model, we showed that once daily administration of recombinant human PTH(1-34) [hPTH(1-34)] at 10 μg/kg per day or 40 μg/kg per day for 140 days activated intracortical remodeling in the tibial midshaft and increased cortical porosity from 1.4% to 6.3%.[11] The increased bone turnover and cortical porosity were accompanied by apposition of bone both periosteally and endocortically, increasing the cross-sectional moment of inertia and resulting in significantly greater bone strength, stiffness, and work to failure in the higher dose group compared with controls. In a subsequent article,[12] we showed that one reason the increased cortical porosity did not have a detrimental effect on mechanical properties was that most of the increase occurred near the endocortical surface, where its mechanical effect was small and more than offset by apposition to the periosteal surface. This showed that the distribution of cortical porosity, which cannot be assessed by noninvasive measurements of bone density, is critical in evaluating biomechanical bone strength.

The rabbit study was limited in duration (140 days, ~2 remodeling periods) and it may be that porosity over a more prolonged period of 4–6 remodeling cycles will impair biomechanical properties of bone. In addition, the consequences of withdrawing PTH treatment have not been studied in animal models with osteonal cortical bone.

The current experiment was designed to study the consequences of more prolonged treatment and the sequelae of withdrawing treatment. Adult ovariectomized (OVX) cynomolgus monkeys were treated with hPTH(1-34) LY333334 for 18 months (~7.5 remodeling periods)[13] or for 12 months (~5 remodeling periods) and then treated with vehicle for the remaining 6 months (~2.5 remodeling periods). Histomorphometric variables and porosity were measured in the left humerus, and the right humerus was mechanically tested. We hypothesized that treatment with recombinant human PTH(1-34) LY333334 would significantly increase porosity in the midshaft of the humerus, but that this would not have a significant effect on the strength or stiffness of the humerus. We also hypothesized that withdrawal of PTH for 6 months after a 12-month treatment period would result in a normalization of porosity and bone turnover to control values.

MATERIALS AND METHODS

Natural habitat-derived adult female cynomolgus monkeys (*Macaca fascicularis*) were imported from Indonesia and quarantined for 3 months. Monkeys were screened radiographically to ensure absence of open growth plates and skeletal abnormalities that might interfere with bone mass measurements. Monkeys were sorted at baseline according to body weight, spine bone mineral density (BMD), and serum alkaline phosphatase levels into one of six treatment groups (Table 1). The sham OVX group (sham, $n = 21$) and one OVX group (OVX, $n = 22$) received 0.1 ml/kg per day of 0.9% saline containing 20 mM NaH_2PO_4 as vehicle control. Two OVX experimental groups received once daily subcutaneous (sc) injections of recombinant hPTH(1-34) LY333334 (synthesized by Eli Lilly & Co., Indianapolis, IN, USA) at doses of 1.0 μg/kg per day (PTH1, $n = 42$) or 5.0 μg/kg per day (PTH5, $n = 43$) starting the day after OVX for 12 months. The lower dose is approximately equivalent to[14] or only slightly higher than[15,16] doses that have been used in recent successful human clinical trials. After 12 months of treatment, PTH treatment was withdrawn from approximately half of the monkeys in each treatment group (PTH1-W, $n = 21$; PTH5-W, $n = 21$), and they were treated for the remaining 6 months with once daily sc vehicle injections. Seven monkeys did not complete the study because of either trauma or disease unrelated to treatment. The beginning and final sample sizes for each group are listed in Table 1. Monkeys were fed a purified diet containing 0.3% Ca, 0.3% P, 29.5% protein, 55% carbohydrate, 4.9% fat, and 9% fiber. Their vitamin D status was normal and did not change during the study. Water was provided ad libitum. A more detailed description of the experimental design and treatment regimens can be found in the work by Brommage.[17]

Recombinant hPTH(1-34) LY333334 was synthesized at Lilly Research Laboratories (Indianapolis, IN, USA) using recombinant DNA technology and was stored lyophilized at −20°C. Doses were prepared daily in plastic CZ vials (West Co., Lionville, PA, USA) in a sterile vehicle of saline containing 20 mM NaH_2PO_4.

Double fluorochrome labels were given at 6 months (tetracycline HCl, 25 mg/kg, intravenously [iv]), 15 months (alizarin complexone, 20 mg/kg, iv), and before death at 18 months (calcein, 10 mg/kg, iv) using a 1–12-1 schedule, with death 7 days after administration of the final label. In this article, parameters are reported only for the 18-month time period because porosity and mechanical properties of the bone could be measured only at the end of the experiment.

Histomorphometry

Static and dynamic histomorphometric measurements of cortical bone from the humeral middiaphysis were performed on the periosteal, endocortical, and intracortical surfaces of cortical bone from the middiaphysis of the left

TABLE 1. EXPERIMENTAL DESIGN

Group	*Abbreviation*	*Monkeys at outset (n = 128)*	*Monkeys in final analyses (n = 121)*
Sham ovariectomized, 18 months vehicle	sham	21	21
Ovariectomized, 18 months vehicle	OVX	22	20
Ovariectomized, 18 months 1 μg LY333334/kg per day	PTH1	21	19
Ovariectomized, 18 months 5 μg LY333334/kg per day	PTH5	22	21
Ovariectomized, 12 months 1 μg LY333334/kg per day, 6 months vehicle	PTH1-W	21	20
Ovariectomized, 12 months 5 μg LY333334/kg per day, 6 months vehicle	PTH5-W	21	20

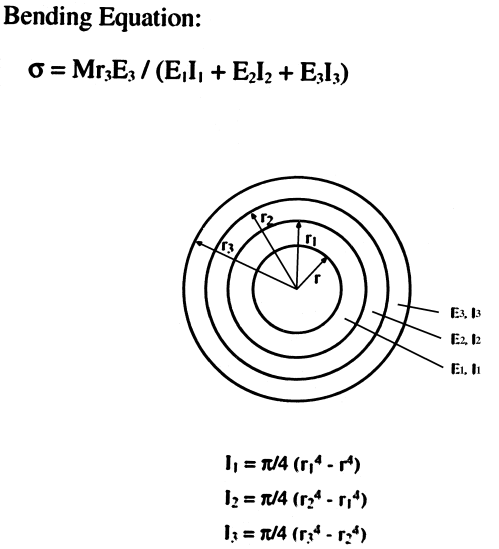

FIG. 1. Intracortical porosity in the humerus was determined in three zones of varying distance from the marrow cavity. The values of r_1, r_2, and r_3 correspond to the radii from the center of the cross-section to the edge of zones I, II, and III, respectively. By estimating how the porosity would change the Young's modulus in each zone, an estimate of strength (θ) was made using composite beam theory. In the bending equation, M = the applied bending moment.

humerus. After death, the bones were removed and preserved in formalin for 1 week and then transferred to 70% alcohol for shipment from Wake Forest University (Winston-Salem, NC, USA) to the Musculoskeletal Research Laboratory (Indianapolis, IN, USA). When received, the bones were fixed in 10% cold neutral-buffered formalin for 5 days. The tissues were dehydrated in a graded series of alcohols (70–100%, two changes per grade, each for 4 h under vacuum). The specimens were then placed in xylene and infiltrated with methylmethacrylate under vacuum at 15 in Hg on a 2-h/step and 24-h infiltration cycle in a Shandon Hypercenter automatic tissue processor (Shandon Lipshaw, Pittsburgh, PA, USA). The specimens were embedded in methylmethacrylate. Sections 80 μm thick were cut using a diamond wire saw (Hisiosaw, Delaware Diamond Knives, Wilmington, DE, USA) and mounted on glass slides. Sections for analysis of fluorochrome labels were left unstained; those measured for porosity were stained with Goldner's trichrome.

Histomorphometry was done at $\times$150 magnification using a Nikon fluorescence microscope (Optiphot; Nikon, Tokyo, Japan) and a semiautomatic digitizing system (Bioquant IV; R&M Biometrics, Nashville, TN, USA). Bone area (B.Ar), medullary area (Me.Ar), cortical area (Ct.Ar = B.Ar $-$ Me.Ar), and bone formation rates on both periosteal (Ps.BFR/BS) and endocortical (Ec.BFR/BS) surfaces were measured from the entire cross-section. Cortical thickness (Ct.Th) was measured in each of the four quadrants of the humeral cross-section using a digital caliper (Mitutoyo Corporation America, Aurora, IL, USA), accurate to 10 $\pm$ 5 μm. The measurements were averaged to obtain mean Ct.Th. Nomenclature follows standards set by the American Society for Bone and Mineral Research (ASBMR) committee on histomorphometric nomenclature.[18]

Ct.Ar and pore area (Po.Ar) were measured by point counting at $\times$150 magnification. Po.Ar included all holes except for osteocyte lacunae and canaliculi. From these variables, cortical porosity (Po, %) was calculated (Po.Ar/Ct.Ar). To evaluate the distribution of cortical porosity, cortical B.Ar was divided into three zones. Zone I was the inner (endocortical) one-third of the cortical diameter near the endocortical surface. Zone II was the intermediate one-third, and Zone III was the outer (periosteal) third of the cortical diameter (Fig. 1). To determine these, Ct.Th was measured microscopically and the thickness was divided equally into three parts. Then, a part of the endocortical surface was selected and a microscopic field was placed along this surface. Ct.Ar and Po.Ar in each zone were measured by point counting from the endocortical to periosteal surface. After finishing the first line, the microscopic field was moved to the second starting point adjacent to the first starting point, as described previously.[12] The same procedure was repeated to cover the entire endocortical

surface. Although the entire cortical B.Ar was not covered by this method, about 65% of the B.Ar was covered. Stereologically, this provides a sufficient sampling to estimate the distribution of cortical porosity in the three zones.

Six sections, equally divided between sham and OVX groups, were measured three separate times by the same observer to calculate the precision of the histomorphometric measurements. Precision was determined as (SD/mean) * 100.

The reduction in strength effected by a change in the distribution of porosity can be calculated by estimating the change in Young's modulus of each region of the bone from the porosity measurements. It was assumed that the Young's modulus of each region of the bone cross-section was proportional to the bone volume fraction $(1 - p)$[19] and density (ρ) was calculated as

$$\rho = \rho_0(1 - p), \quad (1)$$

where ρ_0 is the density of the sham group and p is the porosity. Maximum stress within the humerus for a given load was calculated as

$$\sigma = Mr_3E_3/(E_1I_1 + E_2I_2 + E_3I_3) \quad (2)$$

where M is the bending moment, r is the distance from the center of the cross-section to the edge of each zone (Fig. 1), E is the estimated Young's modulus for each zone, and I is the moment of inertia for each zone. As porosity increases, so will stress and thus strength will be proportionally decreased. The strength reduction was calculated as

$$\%\text{strength reduction} = (\sigma_{\text{sham}} - \sigma_x)/\sigma_{\text{sham}}\} * 100, \quad (3)$$

where σ_{sham} is the stress in the sham group and σ_x is the stress in a given treatment group. The reduction in strength based on measured porosity gradient, and the reduction in strength that would have occurred if the porosity were distributed uniformly throughout the cross-section were compared to each other.

Biomechanical testing

Bone mechanical properties were measured in the right humeral middiaphysis. Bones were resected, cleaned of connective tissue, wrapped in gauze soaked in isotonic saline, and frozen at −20°C until testing. Before testing, specimens were thawed for 1–2 h at room temperature. Humeral bone strength was measured at the midshaft using three-point bending. The humerus was positioned on a fixture with the medial side facing toward the loader. Load was applied at the midpoint between two supports that were 54 mm apart. The bone specimen was displaced at the rate of 1 mm/s until failure occurred. All specimens were tested to failure in a circulating water bath at 37°C using an MTS 810 servohydraulic testing machine (MTS Corp., Minneapolis, MN, USA). Load-deformation curves were recorded using the HP 7090A measurement plotting system (Hewlett-Packard, Carnas, WA, USA). Ultimate force (F_u, the maximum force sustained by a specimen before failure), stiffness (S, the slope of the linear portion of the load-deformation curve), and work to failure (U, area under the load-deformation curve before failure) were measured using a digitizer system (Jandel Scientific, Corte Madera, CA, USA). These measurements are structural parameters that depend both on the intrinsic material properties of the tissue and on the geometry.[20]

TABLE 2. BODY WEIGHT

Group	*Weight at baseline (kg)*	*Weight at 18 months (kg)*
Sham	2.75 ± 0.09	3.00 ± 0.13
OVX	2.66 ± 0.07	2.87 ± 0.10
PTH1	2.78 ± 0.08	2.88 ± 0.07
PTH1-W	2.83 ± 0.07	2.97 ± 0.09
PTH5	2.73 ± 0.05	2.99 ± 0.11
PTH5-W	2.71 ± 0.07	2.91 ± 0.09

Mean ± SEM. There were no significant differences among groups.

Statistical analysis

Variables that were not distributed normally were transformed using either log or square root transformations. In some cases, more than 25% of the groups were nonnormally distributed and either the transformation was ineffective or a Levene's test indicating the inhomogeneity of variance was not corrected by transformation. Differences among groups were assessed using one-way analysis of variance (ANOVA). For variables showing significant differences, post hoc tests were performed with Bonferroni adjustments. The values of $p < 0.05$ were considered significant. The reduction of strength in each treatment group was compared with sham controls or OVX under either the assumption of a porosity gradient such as the one measured or the assumption of uniform porosity, using ANOVA followed by Fisher's projected least significant difference (PLSD) for post hoc comparisons.

RESULTS

Body weight

There were no statistically significant differences in body weight among groups either at baseline or after 18 months of treatment (Table 2).

Calculation of measurement precision

The precision of porosity measurements ranged between 10.76% and 11.67% for the three zones. The precision of cortical surface measurements was between 0.50% (total area [Tt.Ar]), 0.87% (Me.Ar), and 0.47% (Ct.Ar). Precision for the other histomorphometric variables ranged between 3.67% (activation frequency [Ac.F]) and 12.31% (resorption number [Rs.N/Ct.Ar]).

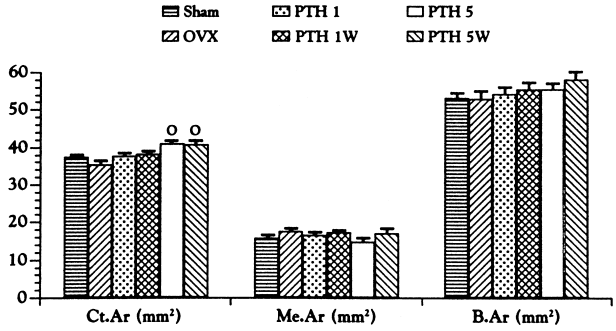

FIG. 2. Ct.Ar increased significantly in PTH5 and PTH5-W compared with OVX. There were no differences among groups in Me.Ar or Tt.Ar of bone within the periosteal surface (B.Ar). O, $p < 0.05$ versus OVX. Data are expressed as mean ± SEM.

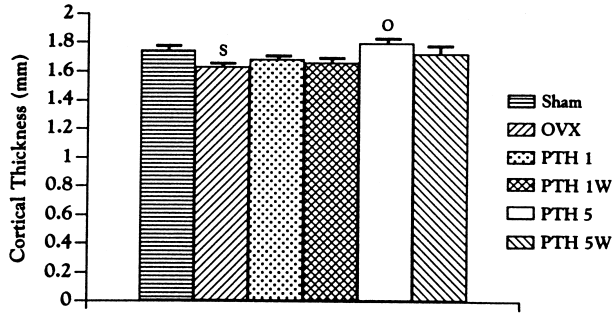

FIG. 3. Ct.Th was significantly less in OVX than in sham and significantly greater in PTH5 than in OVX. S, $p < 0.05$ versus sham; O, $p < 0.05$ versus OVX. Data are expressed as mean ± SEM.

Static parameters

Both higher dose groups (PTH5 and PTH5-W) had significantly more Ct.Ar than OVX monkeys ($p < 0.01$; Fig. 2). There were no differences in Ct.Ar among the other groups. Withdrawal of LY333334 for 6 months after a 12-month treatment period did not result in a reversal to sham control values. There were no significant differences among groups for total area (Tt.Ar) within the periosteal surface (total bone area, B.Ar) or for Me.Ar. Ct.Th was significantly less in OVX than in sham ($p < 0.05$) and significantly greater in PTH5 than in OVX ($p < 0.05$; Fig. 3).

Intracortical remodeling

Compared with sham, Ac.F and intracortical bone formation rate (BFR/BV) were increased significantly in OVX ($p < 0.001$), PTH1 ($p < 0.001$), and PTH5 ($p < 0.001$). There were significantly more resorption spaces (Rs.N/Ct.Ar) in OVX monkeys than in sham ($p < 0.0 1$) and significantly more in PTH 1 and PTH5 than in OVX ($p < 0.001$; Table 3). After PTH treatment was withdrawn for 6 months, all values decreased to sham levels irrespective of dose.

Six months after PTH withdrawal, Ac.F was equivalent to sham values in both PTH1-W and PTH5-W and significantly lower than OVX. Likewise, BFR/BV was similar to sham values in PTH5-W but was significantly elevated ($p < 0.01$) in PTH1-W compared with sham.

Porosity was increased significantly by OVX ($p = 0.003$). Treatment with the lower dose of PTH did not increase porosity over values found in OVX, but porosity significantly increased ($p < 0.001$) compared with sham controls (Fig. 4). Treatment with the higher dose of PTH (PTH5) increased intracortical porosity 3-fold compared with OVX ($p < 0.001$) and 6-fold compared with sham ($p < 0.001$). After withdrawal of treatment, porosity in PTH1-W declined to sham values, significantly lower than OVX, whereas porosity in PTH5-W remained significantly elevated compared with either OVX or sham ($p < 0.001$).

Most of the increase in porosity occurred in zone 1, close to the endocortical surface (Fig. 5). In this region, porosity was increased in PTH5 by about 5-fold compared with sham and OVX controls ($p < 0.05$). In PTH1, porosity was increased 3-fold ($p < 0.05$). Porosity also was increased significantly in zone II and zone III in the PTH treatment groups compared with either sham or OVX ($p < 0.05$), but the magnitude of increase was less compared with that of zone I.

The effects of the porosity gradient on strength were compared with strength reductions that would have occurred if the porosity were uniformly distributed throughout the cross-section. The results show that we would predict a nonsignificant 11% reduction in humeral strength with PTH5 given the porosity gradient that was measured (Fig. 6). If the porosity had been distributed uniformly, strength would have been reduced significantly ($p < 0.05$) by 22%. A uniform porosity distribution also would have caused a significant reduction in strength in PTH1 ($p < 0.05$), but a gradient of porosity such as the one measured would not be predicted to reduce strength significantly in this group.

Mechanical properties

Consistent with the predictive models based on the actual porosity distribution, no significant differences were detected among groups for the extrinsic (i.e., structural) mechanical parameters of ultimate force, stiffness, or work to fracture (Fig. 7).

DISCUSSION

These data show that, in an animal model with Haversian remodeling, PTH treatment significantly increases intracortical porosity by activating new Bone Multicellular Units, but the increased porosity does not have a detrimental effect on the strength, stiffness, or work to fracture of the humerus. These data are important in showing that the decreased bone density previously reported in some human trials of PTH treatment[1,3,21] cannot be assumed to predict a loss of bone strength or increased risk of fracture in those studies.

TABLE 3. INTRACORTICAL MEASUREMENTS OF THE HUMERUS AT 18 MONTHS

Variable	*Sham*	*OVX*	*PTH1*	*PTH1-W*	*PTH5*	*PTH5-W*
Rs.N/Ct.A (/mm^2)	0.12 ± 0.04	0.21 ± 0.03†	0.28 ± 0.04*	0.12 ± 0.02	0.45 ± 0.05*§	0.17 ± 0.04
Ac.F (cycles/year)	1.85 ± 0.43	6.06 ± 0.76*	7.69 ± 1.14*	3.17 ± 0.49†§	9.08 ± 0.79*	2.14 ± 0.32‡
BFR/BV (%/year)	2.13 ± 0.50	9.22 ± 1.20*	9.23 ± 1.40*	4.54 ± 0.80†§	13.52 ± 1.20*	2.32 ± 0.40§
O.Wi (μm)	3.77 ± 0.20	4.04 ± 0.21	3.66 ± 0.15	4.03 ± 0.18	3.72 ± 0.11	3.75 ± 0.19

O.Wi, osteoid width.
* $p < 0.001$ versus sham; † $p < 0.01$ versus sham; ‡ $p < 0.001$ versus OVX; § $p < 0.05$ versus OVX.

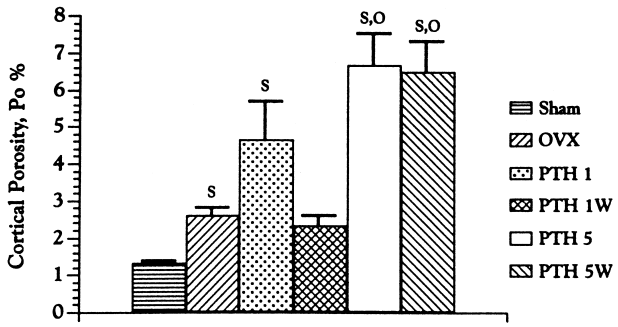

FIG. 4. Intracortical porosity (Po) in the humerus was significantly greater in OVX, PTH1, PTH5, and PTH5-W than in sham. The higher dose groups (PTH5 and PTH5-W) had significantly more intracortical porosity than OVX. S, $p < 0.05$ versus sham; O, $p < 0.05$ versus OVX. Data are expressed as mean ± SEM.

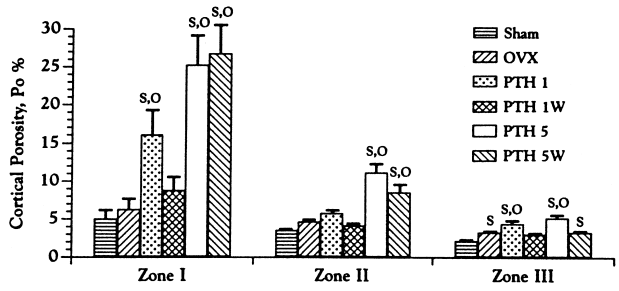

FIG. 5. In the PTH-treated groups, porosity increased most near the endocortical surface (zone I). Porosity was significantly greater in PTH1 and PTH5 than in sham or OVX near the periosteal surface (zone III) as well, but the increases were much smaller. Zone I is the inner (endocortical) one-third of the cortical diameter, zone II is the intermediate one-third, and zone III is the outer (periosteal) one-third of the diameter. S, $p < 0.05$ versus sham; O, $p < 0.05$ versus OVX. Data are expressed as mean ± SEM.

There are two reasons that increased cortical porosity does not translate into decreased mechanical properties. First, most of the porosity was concentrated near the endocortical surface of the cross-section, where its mechanical effect is small. The extrinsic or structural mechanical properties of bone are dependent both on the bone's material properties and on the distribution of bone around a given axis of loading. The bending rigidity of bone is defined by its section modulus (the moment of inertia divided by the radius) and is proportional to the cubed distance from the neutral axis around which bending occurs. Therefore, the loss of bone close to the endocortical surface, which is closest to the neutral axis, has less effect on mechanical properties than if the bone were lost near the periosteal surface. This is clearly shown by comparing the effects of the actual measured porosity gradient with the effects of uniformly distributed porosity on bone strength. If the porosity had been uniformly distributed, strength would have been reduced significantly in both PTH1 and PTH5 compared with sham. Because porosity was distributed to a limited area within the cortex and close to the neutral axis, there were no significant reductions in humeral strength during the active phase of treatment (Figs. 6 and 7).

The second reason that increased porosity is not detrimental to mechanical properties is that there was an increase in the Ct.Ar and Ct.Th in PTH5 (Figs. 2 and 3). This was the result of significantly increased endocortical mineralizing surface in PTH5 compared with sham or OVX controls (Fig. 8). Because the strength of bone is not determined solely by the amount of bone tissue but is also determined by the bone's geometry, an increase in Ct.Ar and Ct.Th will augment the bending rigidity of the bone and offset any potentially detrimental mechanical effects of increased porosity.

The anabolic effect of PTH on endocortical bone surface formation, which leads to increased Ct.Ar and Ct.Th, has been reported widely in both intact[22,23] and OVX rats.[24–27] However, the rat model cannot be used to study the cortical steal phenomenon because of the absence of intracortical remodeling in their bones. For this reason, Mosekilde et al.[27] suggested that the use of a large animal model that has intracortical Haversian remodeling, such as the cynomolgus monkeys used in this study, might be more predictive of putative human responses.

Studies on rats have consistently reported that the bone mass gained during PTH treatment is lost when treatment is withdrawn,[28–32] but no study has shown whether the increased intracortical porosity is reversed when PTH treatment is withdrawn or whether withdrawal alters biomechanical properties. Our data show that at 1 μg/kg per day, the increased porosity is transient and can be reversed with a withdrawal period of only 6 months after a 12-month treatment period. At 5 μg/kg per day, the increased porosity was not reversed with a 6-month withdrawal period. Increased porosity during treatment and return to control values on withdrawal are indicative of a remodeling tran-

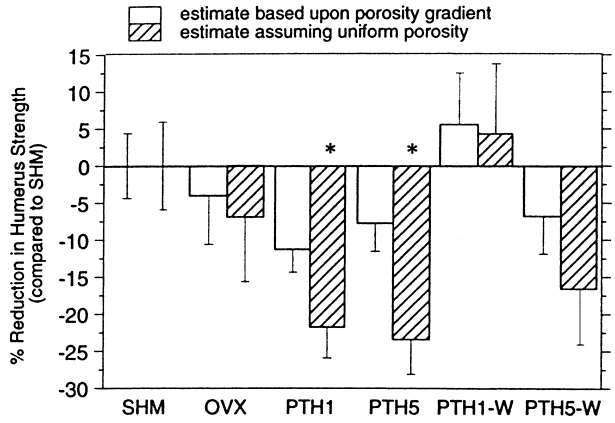

FIG. 6. If porosity were uniformly distributed throughout the cross-section, the reduction in strength of the humerus would have been greater than when the porosity is primarily distributed close to the endocortical surface, as it was in PTH-treated animals. For PTH1 and PTH5, a uniform distribution of porosity would have caused a significant decrease in strength of the humerus ($p < 0.05$). Because there was a gradient of porosity that decreased toward the periosteal surface, no significant reductions in strength were predicted. Data are expressed as mean ± SEM.

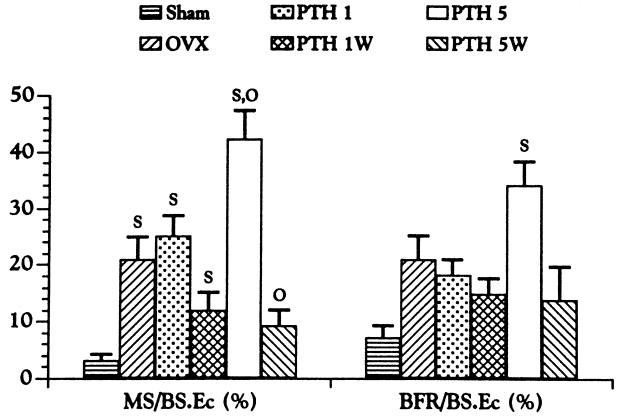

FIG. 8. Mineralizing surface on the endocortical surface of the humerus (MS/BS.Ec) increased significantly in OVX, PTH1, PTH1-W, and PTH5 compared with sham and was significantly greater in PTH5 than in OVX. On withdrawal of PTH for 6 months, MS/BS in the higher dose group declined to significantly less than OVX. Bone formation rate (BFR/BS.Ec) on the endocortical surface increased significantly in PTH5. These changes were responsible for the increased Ct.Ar and Ct.Th found especially in animals given the higher dose of PTH. S, $p < 0.05$ versus sham; O, $p < 0.05$ versus OVX. Data are expressed as mean ± SEM.

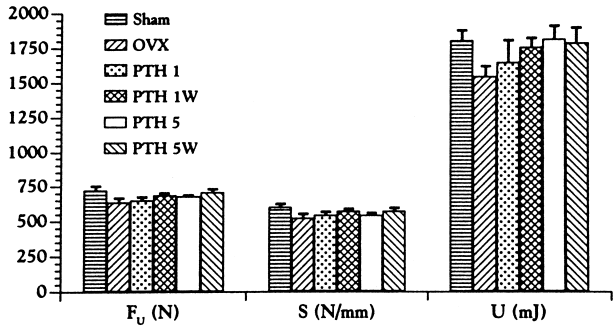

FIG. 7. No significant differences were found among groups for strength (ultimate force, F_u), stiffness (S), or work to fracture (U). Data are expressed as mean ± SEM.

sient that occurs as a consequence of the more rapid Ac.F stimulated by PTH. When treatment is withdrawn, Ac.F returns to normal and the remodeling space is refilled within 2.5 remodeling periods (i.e., 3–6 months) as osteoblasts complete the remodeling process. This supports the notion that resorption and formation are normally coupled in cortical bone in PTH-treated animals and that the increased porosity only reflects a transient state caused by more rapid bone turnover.

The failure of porosity to return to control values on withdrawal in monkeys treated with 5 μg/kg per day may reflect an uncoupling of resorption and formation at these dose levels. Alternatively, it may be that if Ac.F exceeds a certain threshold, a longer withdrawal period is required to complete the remodeling transient.

The results reported here confirm and extend the results obtained using a rabbit model to study the cortical bone effects of PTH treatment. Hirano et al.[11] found that PTH stimulated increased bone turnover and cortical porosity after 140 days of treatment with PTH(1-34), but that the increased porosity was offset by concurrent additions of bone to both periosteal and endocortical surfaces that resulted in an enhancement of the mechanical properties. They determined that in the rabbit model most of the increase in porosity occurred near the endocortical surface where its mechanical effect was small.[12] The nonhomogeneous distribution of porosity was more than offset by the apposition of new bone periosteally, causing an increase in the bending rigidity of the cortical bone. The results reported here for cynomolgus monkeys completely corroborate the findings in the rabbit model, except that additions of new bone in the rabbit model occurred along both the periosteal and the endocortical surfaces, whereas in the monkey model additions of bone were limited to the endocortical surface.

Only two studies using a primate model have measured porosity. Vradenburg et al.[33] reported that daily sc injections of recombinant PTH(1-84) at doses of 1.5 μg/kg per day or 5.0 μg/kg per day, similar to the doses used in the present study, given over a 9-month treatment period increased intracortical porosity by 25% and 50%, respectively in non-OVX rhesus monkeys, increases that were not statistically significant. At a higher dose (10 μg/kg per day), porosity increased by nearly 3-fold, but statistical significance was not achieved. Jerome et al.[34] treated OVX cynomolgus macaques with 10 μg/kg synthetic PTH(1-34) three times per week for 6 months and showed nonsignifi-

cant increases of 20–25% in radial and femoral porosity. Our data show that treatment of OVX cynomolgus monkeys for 18 months with hPTH(1-34) at 1 μg/kg per day or 5 μg/kg per day increased porosity by three to six times compared with vehicle-treated controls, but without any detrimental effect on mechanical properties. Mechanical properties of cortical bone were not measured in the other studies.

None of the studies on animals with Haversian remodeling have reported the distribution of porosity, which appears to be critical to the prediction of changes in bone strength and stiffness in animals and humans treated intermittently with PTH(1-34); they also did not report mechanical properties of the bone after withdrawal of the treatment. Our data are important, showing that although intermittent PTH(1-34) treatment significantly increases intracortical bone turnover, as measured by Ac.F and BFR/BV and intracortical porosity, the increased porosity does not reduce the strength or the stiffness of cortical bone. Moreover, at a lower dose, the effect on intracortical porosity is transient and can be reversed with a short duration of withdrawal of the drug.

We conclude that the increases in intracortical porosity induced by intermittently administered PTH(1-34) does not reduce the strength or stiffness of the cortical bone. The effects of increased porosity are offset both by the distribution of the porosities close to the endocortical surface and by the increased endocortical mineralizing surface that results in a significantly greater average Ct.Th in animals treated at the higher dose. We further conclude that the normalization of porosity after a period of withdrawal of PTH treatment is dose dependent; at the lower dose normalization occurred relatively rapidly, but at the higher dose porosity does not return to normal after 6 months following a 12-month treatment period presumably because the increased rate of turnover requires a long period to reverse itself. If the biomechanical properties of bone are predictive of resistance to fracture, data from the OVX monkey model suggest that PTH will alter geometric properties of cortical bone but will not pose an additional fracture risk during or after treatment.

ACKNOWLEDGMENTS

This study was funded by Eli Lilly & Co. Technical assistance from Ricky Cain, Taffy Hooser, Diana Jacob, and Thurman Alvey is gratefully acknowledged. Phil Iversen, from Lilly Research Laboratories, performed the statistical calculations.

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Received in original form December 7, 1999; in revised form April 17, 2000; accepted May 30, 2000.