

Teriparatide [PTH(1-34)] Strengthens the Proximal Femur of Ovariectomized Nonhuman Primates Despite Increasing Porosity

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ABSTRACT: OVX monkeys treated for 18 months with 1 or 5 $\mu\text{g}/\text{kg}/\text{d}$ teriparatide [PTH (1-34)] had significantly stronger proximal femora relative to ovariectomized controls. Teriparatide enhancement of cortical area, cortical width, and trabecular bone volume seemed to more than compensate for the dose-dependent increase in cortical porosity. Beneficial effects of teriparatide treatment on the proximal femur persisted beyond the treatment period and may extend to the marrow.

Introduction: We conducted a detailed quantitative analysis of the effects of teriparatide on the proximal femur of ovariectomized monkeys. Teriparatide increased bone mass, enhanced structural architecture, and strengthened the hip, despite increasing cortical porosity.

Materials and Methods: Monkeys were treated with vehicle (sham or OVX controls), 1 $\mu\text{g}/\text{kg}/\text{day}$ teriparatide [parathyroid hormone (1-34); PTH1], or 5 $\mu\text{g}/\text{kg}/\text{day}$ teriparatide (PTH5) for 18 months or for 12 months followed by 6 months of treatment withdrawal (PTH1W and PTH5W, respectively). Excised proximal femora were analyzed by μCT , conventional histomorphometry, and biomechanics.

Results and Conclusions: The femoral neck showed significant reduction in trabecular bone volume (BV/TV) for OVX compared with sham, whereas PTH1 BV/TV was restored to sham levels and PTH5 BV/TV was greater than sham and OVX. The withdrawal groups had BV/TVs intermediate between sham and OVX. PTH1 had trabecular number (Tb.N) greater than OVX, and PTH5 Tb.N was greater than sham and OVX. The withdrawal groups had Tb.Ns intermediate between sham and OVX. No differences between groups were observed for trabecular orientation or trabecular thickness. Teriparatide dose-dependently increased bone formation rate and activation frequency in the femoral neck. Cellular composition analyses suggested a tendency of ovariectomy to increase adiposity of marrow by 100%, whereas PTH tended to reduce adipocyte number and increase osteoblast number compared with OVX. Analyses of the cortex showed dose-dependent elevation of cortical porosity, which was consistent with enhanced bone turnover with treatment. Cortical porosity was reduced after withdrawal of teriparatide, because PTH1W cortical porosity was lower than OVX, whereas PTH5W cortical porosity was intermediate between sham and OVX. Increased cortical porosity did not weaken the proximal femora. Biomechanics showed that ovariectomy weakened proximal femora compared with sham, but PTH1, PTH5, and PTH1W were stronger than OVX and not different from sham. PTH5W strength was intermediate between sham and OVX. Therefore, teriparatide had beneficial effects on the proximal femur, despite increasing cortical porosity. Cortical porosity did not adversely affect the mechanical integrity of the proximal femora, because enhanced cortical area and trabecular bone volume more than compensated for the porosity. Much of the beneficial effects of teriparatide were retained after 6 months withdrawal from treatment. PTH effects on the femoral neck were not limited to bone but may include inhibition of OVX-stimulated adiposity of the marrow.

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Key words: parathyroid hormone, recombinant human parathyroid hormone (1-34), biomechanics, bone histomorphometry, μCT , proximal femora

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INTRODUCTION

TERIPARATIDE [human parathyroid hormone (PTH)(1-34)] is a new therapeutic option for osteoporosis that has been shown to induce new bone formation onto

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trabecular and cortical surfaces in monkeys, women, and men.⁽¹⁻³⁾ Previous studies showed that PTH strengthens vertebra in monkeys and humans, resulting in reduced rates of vertebral fractures in postmenopausal women with osteoporosis.⁽⁴⁻⁶⁾ In addition, teriparatide reduced the risk of nonvertebral fragility fractures by 53–54% in a large, double-blind, placebo-controlled clinical trial.⁽⁶⁾ While these data are encouraging, efficacy in a clinical hip fracture trial has not been explicitly demonstrated because of insufficient patient enrollment and low incidence of hip fractures in previous clinical studies.^(2-4,6) Therefore, questions persist as to the effects of PTH on the hip of primates.

The proximal femur is a mixed trabecular and cortical bone site, consisting of mostly trabecular bone and about 12% cortical bone.⁽⁷⁾ Biomechanical analyses have shown that cortical bone is disproportionately more important than trabecular bone in contributing to hip strength.⁽⁸⁾ Unfortunately, PTH has been shown to have controversial effects on cortical bone. Early clinical studies suggested that the trabecular bone effects of PTH occurred at the expense of cortical bone, as evidenced by a reduction in BMD of the diaphysis of long bones.^(6,9) In addition, teriparatide was shown to increase cortical porosity as a result of enhanced rate of bone turnover,⁽¹⁰⁾ which is of concern because engineering studies showed that porosity can exponentially compromise strength.⁽¹¹⁾

Analysis of the humerus from ovariectomized (OVX) monkeys showed that teriparatide increased the rate of bone turnover and cortical porosity but did not significantly alter bone mass or biomechanical strength compared with either OVX or sham controls.⁽¹⁰⁾ Part of the explanation for this finding was that intracortical porosity was spatially localized to along the endocortical surface where the mechanical effect is much smaller than bone along the periosteal region.^(10,11) Therefore, the enhanced endocortical porosity was compensated by an increase in cortical area.⁽¹⁰⁾

To clarify the effects of clinically relevant teriparatide administration on the primate hip, we quantitated the strength and micro-architecture of the proximal femur from a primate model shown previously to accurately model clinical efficacy of osteoporosis therapies.⁽¹²⁾ OVX cynomolgus macaques were treated daily with 0, 1, or 5 $\mu\text{g}/\text{kg}$ teriparatide for 18 months; in addition, withdrawal effects from PTH treatment were also evaluated in an effort to ascertain persistence of teriparatide skeletal efficacy in the proximal femur. In the latter case, OVX monkeys were dosed with 0, 1, or 5 $\mu\text{g}/\text{kg}$ teriparatide for 12 months and then switched to vehicle for the remaining 6 months. In a previously described clinical trial,⁽⁶⁾ osteoporotic women were treated for a median duration of 21 months with 20 or 40 μg teriparatide; because women have an average body weight of about 60 kg, clinical doses⁽⁶⁾ used would correspond to monkey doses of about 0.3 and 0.7 $\mu\text{g}/\text{kg}$. Therefore, data from the low dose is likely to be more clinically relevant than the high-dose data.

TABLE 1. FIRST STUDY PROTOCOL

| Group | 12 Months | 18 Months |
|-------|---------------------------|---------------------------|
| Sham | Vehicle | Vehicle |
| OVX | Vehicle | Vehicle |
| PTH1 | 1 $\mu\text{g}/\text{kg}$ | 1 $\mu\text{g}/\text{kg}$ |
| PTH1W | 1 $\mu\text{g}/\text{kg}$ | Vehicle |
| PTH5 | 5 $\mu\text{g}/\text{kg}$ | 5 $\mu\text{g}/\text{kg}$ |
| PTH5W | 5 $\mu\text{g}/\text{kg}$ | Vehicle |

Cynomolgus monkeys were OVX (except for sham) and administered 0, 1, or 5 $\mu\text{g}/\text{kg}/\text{day}$, PTH for 18 months (PTH1, PTH5) or for 12 months followed by 6 months of withdrawal (PTH1W, PTH5W). In addition, a second study was conducted in which 9- to 13-year-old cynomolgus monkeys were OVX and administered 0 or 5 $\mu\text{g}/\text{kg}/\text{day}$ subcutaneous PTH for 18 months.

MATERIALS AND METHODS

Study 1

Adult, cynomolgus monkeys (*Macaca fascicularis*) of ~9 years of age and weighing 2.77 ± 0.03 kg were imported from Indonesia, quarantined for 3 months, randomized, and initially evaluated as described previously.⁽¹³⁾ Animals were OVX (except for sham-OVX controls), and dosed subcutaneously with vehicle (sham and OVX controls), 1 (PTH1), or 5 $\mu\text{g}/\text{kg}/\text{day}$ (PTH5) recombinant hPTH(1-34) (LY333334; Lilly) for 18 months (Table 1). Two additional groups were administered 1 or 5 $\mu\text{g}/\text{kg}/\text{day}$ PTH (PTH1W or PTH5W, respectively) for 12 months and then switched to vehicle for the remaining 6 months (Table 1). Monkeys were injected daily between 9:00 a.m. and 1:00 p.m. with compound in a sterile vehicle of 40 mg/ml mannitol and 20 mM sodium phosphate buffer in saline. Each group consisted of ~20 monkeys. No complications related to treatment were observed, as described previously.⁽¹⁴⁾ Specimens were analyzed after necropsy in a blinded manner during data acquisition.

Study 2

A good laboratory practices (GLPs) study was conducted to evaluate high-dose effects of teriparatide on numerous tissues, including multiple bone sites. In this study, adult, cynomolgus monkeys of ~9–13 years of age were randomized, OVX, and dosed subcutaneously with vehicle (OVX controls) or 5 $\mu\text{g}/\text{kg}/\text{day}$ (PTH5) recombinant hPTH (1-34) (LY333334; Lilly) for 18 months. There were six animals in each group in study 2. Specimens were analyzed after necropsy in a blinded manner during data acquisition. All animal procedures were performed in accordance with federal, state, and institutional guidelines.

Bone labeling and histomorphometry:

Toward the end of study 1, but before necropsy, animals in study 1 were intravenously administered 10 mg/kg calcitonin on a 1–12–1–7 schedule. Animals were necropsied at study termination, and the left proximal femurs were fixed, dehydrated in ethanol, infiltrated with methylmethacrylate, sectioned, and stained for histology as described previously.⁽¹⁴⁾ Femoral neck sections were oriented with a ver-

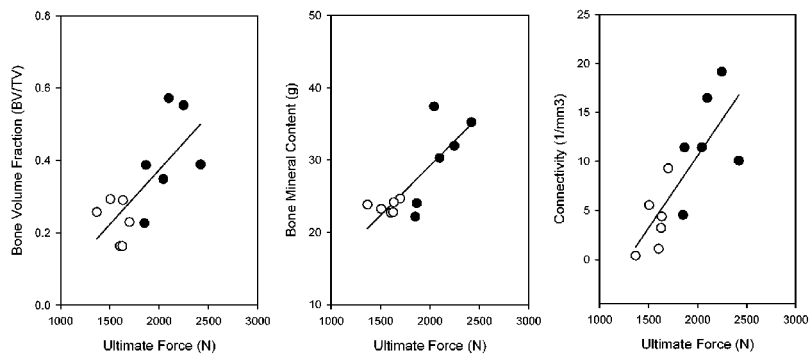


FIG. 1. Linear regression analyses. Regression analyses were conducted in an effort to understand which parameter best correlated with femoral neck strength for study 2 animals, in which the same specimen was analyzed by μ CT before biomechanical testing. \circ , OVX animals; \bullet , PTH5 animals. Linear regression analyses showed significant correlation ($p < 0.015$) of femoral neck strength with BMC ($r = 0.81$), trabecular connectivity ($r = 0.79$), and bone volume fraction (BV/TV, $r = 0.71$).

tical axis that bisected the tissue and passed through the most proximal part of the section.⁽¹⁴⁾ Measurements were made on 4 fields \times 4 fields located centrally in the femoral neck for trabecular bone and of the entire cortical bone region. Measurements included static and dynamic parameters as detailed previously.⁽¹⁴⁾ Animals in study 2 were not administered fluorochrome label.

μ CT

Blocks of the femoral neck from study 1 were analyzed by μ CT, using $15 \times 15 \times 15 \mu\text{m}$ voxels with an EVS μ CT (EVS, London, Ontario, Canada). The intact, left, proximal femur from study 2 was analyzed directly by μ CT, using $36 \times 36 \times 36 \mu\text{m}$ voxels. Femoral neck parameters that were analyzed included total neck volume, neck area, BMD, BMC, trabecular bone volume (BV/TV), trabecular bone surface (BS/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and connectivity.

Load to failure analyses of the proximal femur

The contralateral proximal femora from study 1 were loaded to failure using a servo-hydraulic MTS machine (MTS 810; MTS Corp., Minneapolis, MN, USA) and a load rate of 50 N/s in the laboratory of CT. For study 2, the left proximal femora were loaded to failure using a model 1/S materials testing machine (MTS Corp) in the laboratory of MS. Specimens were placed in a 37°C saline bath for about 3 minutes before testing to allow equilibration of temperature. Load-displacement curves for study 2 were recorded at a crosshead speed of 0.23 mm/s and analyzed using TestWorks 4 software (MTS Corp.). Ultimate load (F_u) for the femoral neck was measured by mounting the proximal half of the femur vertically in a chuck and applying a downward force on the femoral head until failure.⁽⁸⁾ The ultimate load was measured as the maximum force sustained by the femoral neck and considered an estimate of femoral neck strength. Stiffness was calculated as the maximum slope of the load-displacement curve. Energy to break (work to failure) was calculated as the area under the linear portion of the load-displacement curve.⁽⁸⁾

Statistics

Data are presented as mean \pm SEM. Group differences were assessed by ANOVA, with pair-wise contrasts exam-

ined primarily with Fisher's protected least significant difference (PLSD). The significance level for the overall ANOVA was $p < 0.05$ (StatView; Abacus Concepts, Berkeley, CA, USA).

RESULTS

μ CT analysis of the femoral neck

μ CT analysis of the femoral neck (Fig. 1) confirmed that this site is a mixture of trabecular and cortical bone, with thin regions of the cortex that cannot be easily separated into endocortical and periosteal regions, as segmented previously for the humerus.⁽¹⁰⁾ After 18 months, OVX was confirmed to reduce trabecular bone volume by 28% but had no effect on trabecular thickness compared with sham (Table 2). OVX also tended to reduce trabecular number and increase trabecular separation, although these effects were not significant compared with sham (Table 2). Relative to OVX, the low dose of teriparatide (PTH1) increased trabecular bone volume by 33%, trabecular number by 49%, and decreased trabecular separation by 35%. High-dose treatment (PTH5) increased trabecular bone volume by 72% and decreased trabecular separation by 54%, but had no advantage over the low dose in increasing trabecular number relative to OVX. Neither treatment group nor treatment-withdrawal group had any effect on trabecular thickness. The low-dose withdrawal group (PTH1W) had 11% greater trabecular bone volume, whereas the high-dose withdrawal group (PTH5W) had 22% greater trabecular bone volume compared with OVX after 6 months withdrawal from treatment. No significant effects on trabecular number or separation were retained at study termination, although mean values suggested some retention of teriparatide efficacy after treatment withdrawal (Table 2). In specimens from study 2, teriparatide was observed to significantly increase the connectivity of trabeculae in PTH5 compared with OVX (Figs. 1 and 2). The collective data show that teriparatide increased trabecular bone volume and trabecular number and reduced trabecular separation while having no effect on trabecular thickness (Table 2).

Conventional histomorphometry and porosity analyses of the cortex

Conventional histomorphometry was conducted to clarify bone formation activity, resorption activity, and the rate of

TABLE 2. μ -CT ANALYSIS OF THE FEMORAL NECK AFTER TERIPARATIDE TREATMENT

| Group | Trabecular bone volume | Trabecular thickness (mm) | Trabecular number (#/mm) | Trabecular separation (mm) |
|-------|------------------------|---------------------------|--------------------------|----------------------------|
| Sham | 0.23 \pm 0.03* | 0.101 \pm 0.007 | 2.26 \pm 0.22 | 0.39 \pm 0.05 |
| OVX | 0.18 \pm 0.01 | 0.104 \pm 0.004 | 1.73 \pm 0.10 | 0.57 \pm 0.09 |
| PTH1 | 0.24 \pm 0.02* | 0.095 \pm 0.005 | 2.58 \pm 0.35* | 0.37 \pm 0.07* |
| PTH1W | 0.20 \pm 0.14 | 0.102 \pm 0.009 | 2.02 \pm 0.22 | 0.50 \pm 0.07 |
| PTH5 | 0.31 \pm 0.04* | 0.101 \pm 0.003 | 2.58 \pm 0.35* | 0.26 \pm 0.04* |
| PTH5W | 0.22 \pm 0.15 | 0.096 \pm 0.005 | 2.28 \pm 0.14 | 0.38 \pm 0.03 |

Trabecular bone architecture of the femoral neck were analyzed in 3D by μ CT.

* Significant differences with respect to OVX ($p < 0.05$, Fisher's PLSD).

Teriparatide significantly increased trabecular bone volume, trabecular number, and decreased trabecular separation compared with OVX but did not alter trabecular thickness.

TABLE 3. DYNAMIC HISTOMORPHOMETRY OF THE FEMORAL NECK CORTEX

| Group | Osteoid.Os (%) | Form.Os (%) | Resorp.Os (%) | Os.BFR/BS (μ m/dx100) | Os.Act.F (#/day) |
|-------|----------------|-----------------|----------------|----------------------------|--------------------|
| Sham | 11.3 \pm 1.9 | 30.2 \pm 5.1 | 8.9 \pm 1.7 | 8.1 \pm 2.0 | 0.015 \pm 0.003 |
| OVX | 15.4 \pm 3.4 | 37.5 \pm 5.5 | 11.7 \pm 1.8 | 8.3 \pm 1.4 | 0.018 \pm 0.004 |
| PTH1 | 13.8 \pm 2.2 | 39.8 \pm 5.0 | 7.6 \pm 1.3 | 13.0 \pm 2.4 | 0.031 \pm 0.006 |
| PTH1W | 6.6 \pm 1.6* | 18.8 \pm 4.8* | 6.8 \pm 0.8* | 6.0 \pm 1.6 | 0.013 \pm 0.005 |
| PTH5 | 21.7 \pm 3.8 | 42.2 \pm 6.0 | 10.0 \pm 1.8 | 15.3 \pm 2.5* | 0.059 \pm 0.015* |
| PTH5W | 7.6 \pm 2.1* | 21.9 \pm 4.5 | 7.9 \pm 1.6 | 6.5 \pm 2.1 | 0.020 \pm 0.006 |

Bone formation activity and the rate of turnover of the femoral neck cortex were evaluated by conventional histometry.

* Significant differences with respect to OVX ($p < 0.05$, Fisher's PLSD).

Cortical bone histomorphometry of the femoral neck showed an increase in osteon bone formation rate (Os.BFR/BS) and osteon activation frequency (Os.Act.F) with PTH treatment. Percent osteon osteoid surface (Osteoid.Os), forming osteons, and resorbing osteons declined below OVX after 6 months withdrawal, especially for PTH1W.

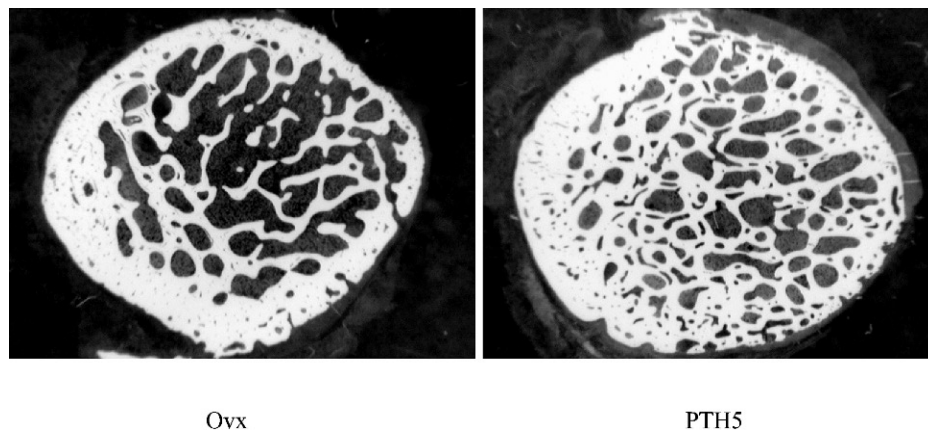


FIG. 2. μ CT images of the femoral neck after 18 months of treatment. Teriparatide treatment (right) seemed to increase trabecular area and trabecular number. Treatment seemed to increase tunneling of thickened trabeculae and the cortex, which was not different from OVX (left) with withdrawal, especially for PTH1W. Teriparatide also increased cortical width and area in femoral neck.

bone turnover in the cortical bone of the femoral neck (Table 3). After 18 months, no differences in percent osteon osteoid surface (Osteoid.Os), forming osteons (Form.Os), resorbing osteons (Resorp.Os), osteon bone formation rate (Os.BFR/BS) or osteon activation frequency (Os.Act.F) were observed between sham and OVX controls (Table 3). Teriparatide dose-dependently increased osteon activation frequency (Os.Act.F) by 228% and osteon bone formation rate (Os.BFR/BS) by 84%, and tended to increase Osteoid.Os compared with OVX. Withdrawal from treatment for PTH1W lowered forming osteons (Form.Os) by 50%, resorbing osteons (Resorp.Os) by 42%, and Osteoid.Os by

57% compared with OVX. Therefore, teriparatide treatment tended to improve the ratio of forming osteons (Form.Os) to resorbing osteon (Resorp.Os) compared with OVX, whereas dynamic parameters tended to return to sham levels or lower after withdrawal, especially for the lower dose (PTH1W).

Images of the femoral neck showed tunneling of thickened trabeculae and increased porosity of the cortex (Fig. 2). Cortical porosity was measured by conventional histomorphometry because of concerns that the spatial resolution used by μ CT may be insufficient to exclude partial volume averaging (Table 4). Intracortical porosity of the femoral neck was not significantly different between sham and OVX

TABLE 4. STATIC HISTOMORPHOMETRY OF THE FEMORAL NECK

| Group | Po.Ar/Ct.Ar (%) | CT.Wi (µm) | Ct.Ar/T.Ar (%) | Adipocytes/Marrow Ar |
|-------|-----------------|------------|----------------|----------------------|
| Sham | 6.0 ± 0.4 | 860 ± 53 | 37.7 ± 2.3 | 15.8 ± 2.7 |
| OVX | 6.7 ± 0.7 | 858 ± 33 | 35.4 ± 1.6 | 36.0 ± 10 |
| PTH1 | 8.5 ± 0.8* | 900 ± 40 | 38.5 ± 2.2 | 16.5 ± 3.3 |
| PTH1W | 4.8 ± 0.5* | 887 ± 48 | 38.5 ± 1.8 | 23.2 ± 4.8 |
| PTH5 | 8.9 ± 0.6* | 1063 ± 31* | 50.8 ± 1.5* | 22.8 ± 6.3 |
| PTH5W | 6.2 ± 0.6 | 967 ± 37 | 44.7 ± 2.1* | 22.0 ± 4.7 |

* Significant differences with respect to OVX ($p < 0.05$, Fisher's PLSD).

Cortical bone histomorphometry of the femoral neck showed an increase in cortical porosity (Po.Ar/Ct.A) with PTH treatment that declined with withdrawal, especially for PTH1W. PTH also increased cortical width (CT.Wi) and cortical area (Ct.Ar/T.Ar). Adipocyte content was also measured for the femoral neck marrow; however, significant differences between groups were not observed because of variance in marrow content between animals and positioning of sections.

after 18 months, but teriparatide increased porosity by 27% for PTH1 and 33% for PTH5 compared with OVX. This condition seemed to be reversible because porosity declined to 28% and 7% below OVX for PTH1W and PTH5W, respectively, after 6 months of withdrawal from treatment. Teriparatide also dose-dependently increased cortical width by 24% and cortical area by 44% for PTH5 relative to OVX. Cortical area remained elevated by 26% for PTH5W compared with OVX after withdrawal from treatment (Table 4).

We also attempted to evaluate teriparatide effects on the cellular composition of the femoral neck. Quantification of adipocyte and osteoblast number showed no significant differences between groups because of problems with variance between monkeys and positioning of sections (Table 4). However, the mean values for adipocytes in OVX marrow were 100% greater than sham in the femoral neck, whereas adipocyte values for PTH1 were 54% lower than OVX after 18 months. Teriparatide also tended to increase osteoblast number (data not shown) in the femoral neck relative to OVX, although the differences were not significant. The histomorphometry data taken together showed that teriparatide increased bone formation activity, rate of bone turnover, and cortical porosity with treatment, which all returned to normal levels after 6 months of withdrawal.

Biomechanical analyses of the proximal femur

To clarify the biomechanical effects of teriparatide, the proximal femur from both studies were loaded to failure after necropsy. OVX for 18 months was observed to weaken the proximal femur by 14% compared with sham (Fig. 3). Teriparatide strengthened the proximal femur by 12% and 23% for PTH1 and PTH5, respectively, compared with OVX. After 6 months of treatment withdrawal, PTH1W was 14% stronger than OVX, whereas PTH5W was 10% stronger than OVX, suggesting some retention of femoral neck strength after discontinuation of teriparatide administration.

Regression analyses were conducted in an effort to ascertain which parameter best correlated with femoral neck strength. The same specimen was analyzed by µCT before biomechanical testing for study 2 animals. Linear regression

Failure Analysis of Proximal Femora

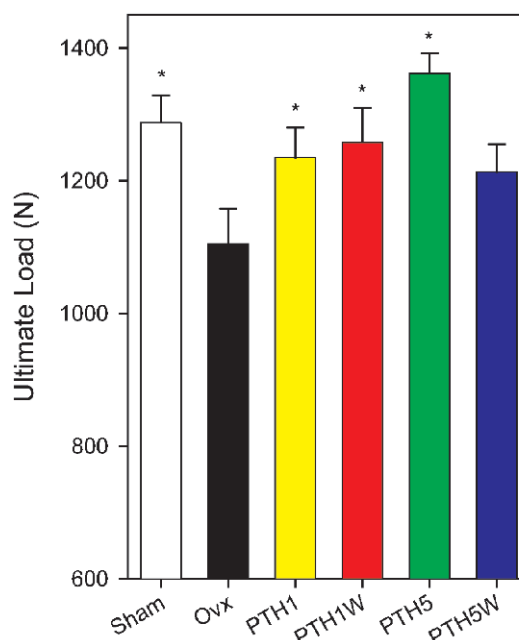


FIG. 3. Biomechanical analysis of the proximal femur. *Significant differences with respect to OVX ($p < 0.05$, Fisher's PLSD). Teriparatide dose-dependently (PTH1, PTH5) increased the strength of the proximal femur compared with OVX, with much of the benefit persisting through withdrawal of 6 months (PTH1W, PTH5W).

analyses showed significant correlation ($p < 0.05$) of femoral neck strength with many parameters, but the best correlations were observed with respect to BMC ($r = 0.81$), trabecular connectivity ($r = 0.79$), and bone volume fraction (BV/TV, $r = 0.71$). These data confirm the importance of both cortical and trabecular compartments in contributing to femoral neck strength in monkeys.

DISCUSSION

Previous studies showed that teriparatide strengthens vertebra in monkeys and humans, resulting in reduced rates of vertebral fractures in postmenopausal women with osteoporosis.⁽⁴⁻⁶⁾ Finite element modeling of monkey vertebra showed that teriparatide induces mineral apposition to reduce strain, thereby decreasing the likelihood of a critical break occurring that could lead to failure.⁽⁵⁾ In postmenopausal women with osteoporosis, teriparatide reduced the risk of nonvertebral fragility fractures by 53-54%; however, questions remain as to the fracture efficacy of PTH on the hip. Recently, beneficial effects of 20 and 40 µg teriparatide on the structural geometry of the hip were shown for women, consistent with improved axial and bending strength as well as theoretical improvements in mechanical strength.^(15,16) However, teriparatide efficacy in reducing hip fractures has not been explicitly demonstrated because of insufficient patient enrollment in early clinical studies and low hip fracture incidence in recent trials.^(2,3,6) The present biomechanical and micro-architectural analysis of

the hip, for OVX monkeys treated with teriparatide, indicates that teriparatide significantly improves hip strength.

Bone strength has been shown to be a complex function of matrix properties and skeletal geometry.^(8,11) Previously, the antiresorptive alendronate was shown to significantly reduce the risk of hip fractures and enhance secondary mineralization by powerfully suppressing bone turnover.⁽¹⁷⁻¹⁹⁾ Increased state of mineralization is the likely explanation for the increased DXA BMD and fracture efficacy observed, which occurred without affecting bone volume or any other architectural parameter in women.⁽¹⁷⁾ Therefore, bisphosphonates seem to function by altering properties of the mineral matrix, increasing the state of mineralization, which was shown previously to increase the strength and stiffness of bones. Unfortunately, the state of mineralization has also been shown to have biphasic effects on bone toughness,⁽²⁰⁾ with possibly adverse effects on brittleness with hypermineralization.⁽¹¹⁾ Therefore, quantitation of both biomechanical properties and micro-architecture seem to be necessary to understand pharmacological efficacy of treatment.

PTH was shown previously to increase bone volume and several architectural parameters but slightly reduce the state of mineralization, which is consistent with activation of osteoblasts to form new packets of bone that is undergoing primary mineralization.^(5,14,21-24) No evidence of any mineralization defect was observed because PTH had no effect on mineralization or remodeling periods in this or in a previous PTH monkey study⁽²²⁾ (C Jerome, personal communication, 2000). However, teriparatide was observed to increase the rate of bone turnover, resulting in increased tunneling of thickened trabeculae and increased intracortical porosity. The former has been attributed to a process by which teriparatide increases trabecular number and connectivity in primates^(14,23,25) (D Dempster, personal communication, 2002). However, intracortical porosity can exponentially compromise material strength,⁽¹¹⁾ and therefore materials analysis was conducted of a beam of pure monkey bone machined from the femur.⁽¹⁰⁾ Curiously, teriparatide had no effect on the material properties of bone tissue and no negative effect on the biomechanical properties of the humerus diaphysis of monkeys.⁽¹⁰⁾ The likely explanation is that the increased porosity was localized toward the endocortical surface where the mechanical contribution is minimal compared with the periosteal region.⁽¹⁰⁾

Unfortunately, we confirmed that regions of the femoral neck are so thin that the cortex cannot be segmented into endocortical and periosteal regions. Nevertheless, biomechanical testing clearly showed that teriparatide strengthens the proximal femur at both doses after 18 months of treatment. An earlier study showed that PTH strengthens the proximal femur of monkeys after 6 months of treatment⁽²²⁾; therefore, PTH seems to strengthen the hip of monkeys after short-term and long-term treatment, as shown previously for rabbits.^(26,27) A likely explanation is that teriparatide increased cortical area and cortical thickness, which more than compensated for the increased porosity, resulting in stronger hips in OVX cynomolgus macaques. Teriparatide also dose-dependently increased the trabecular bone volume, trabecular number, and reduced trabecular spacing in the femoral neck. Correlation analyses of proximal femur

strength with respect to trabecular bone volume and connectivity showed that the structural contributions of trabecular micro-architecture to hip strength are important, in addition to cortical bone properties.

Much of the teriparatide effects on the proximal femur were retained after 6 months of withdrawal including enhanced strength, cortical area, trabecular bone volume, trabecular number, and reduced cortical porosity. Therefore, the beneficial effects of teriparatide extended beyond the treatment period as the rate of bone turnover returned toward normal levels. Actually, because cortical porosity was reduced to significantly below OVX with 6 months of withdrawal, the ideal treatment regimen with teriparatide might include a period of withdrawal after transient administration. Additional studies are necessary to clarify what happens with longer periods of withdrawal from teriparatide treatment.

PTH was shown previously in rats to reduce the adipocyte content of the marrow while increasing osteoblast number.^(28,29) We found similar trends, although significance was not attained because of variance in the cellular content of the femoral neck between monkeys. Nevertheless, the data taken together do indicate that teriparatide does help preserve red marrow by stimulating stromal cell differentiation away from adipocytes toward osteoblasts in primates, as well as in rats.^(28,29) PTH preservation of red marrow may help to explain the rapidity of skeletal efficacy and suggest additional physiological benefits beyond skeletal tissue and the treatment phase.

In summary, teriparatide had beneficial effects on the skeletal mass, structural architecture, and biomechanical integrity of the hip from OVX monkeys, despite increasing cortical porosity. Porosity was not observed to adversely affect mechanical properties, because enhanced cortical area, cortical width, and trabecular bone volume of the proximal femora more than compensated for the porosity, resulting in stronger hips. In addition, analysis of cellular composition suggest that teriparatide effects on the femoral neck may not be limited to activation of osteoblasts, but may include inhibition of OVX-stimulated adiposity of the marrow. Finally, these data point to the important advantage of nonhuman primate models over clinical trials where detailed quantitative analyses can be conducted of pharmacological efficacy for the desired site.

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