

Anabolic actions of PTH in the skeletons of animals

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Abstract

A brief historical perspective reviews studies that tested the hypotheses that PTH induces an anabolic effect in bone, and that the gain in trabecular bone was not at the expense of cortical bone. As PTH reduces the risk of fracture in humans with osteoporosis, the myths that postulated cortical bone porosity and increased bone turnover might increase fracture risk, are examined in the light of data from animals with osteonal bone. These show that PTH “braces” the bone by immediately stimulating bone formation at modeling and remodeling sites. Increased porosity is a late event, occurring close to the neutral axis of bone where detrimental effects on biomechanical strength are unlikely. PTH increases bone mass by stimulating modeling in favor of bone formation, and restructures bone geometry via more extensive remodeling. Cell and genetic events induced in bone by PTH have been studied in rats and are time- and regimen-dependent. In addition to the stimulation of gene expression for matrix proteins, early genes upregulated by once daily PTH are those associated with matrix degradation and induction of osteoclastic resorption, indicative of possible mechanisms by which PTH may increase bone turnover. Bone-forming surfaces are increased due to increased numbers of newly differentiated osteoblasts and retention of older osteoblasts by inhibition of apoptosis. After stopping treatment, the number of osteoblasts is quickly reduced and bone turnover returns to that of controls, slowing both bone formation and resorption. The increased proportion of bone undergoing PTH-induced remodeling requires maturation and completion of mineralization. These responses may explain the delay in reversal of gains in bone mass and biomechanical properties for at least two turnover cycles following withdrawal in large animal models. Thus, the skeletal benefits of PTH extend beyond the active treatment phase.

Keywords: Parathyroid Hormone, Bone Development, Bone Turnover, Cortical Porosity, Osteoblasts

“The effects of the (parathyroid) hormone differ somewhat with the species of animal, the state of the existing parathyroid function, the dose, the mode of administration, and the composition of the diet”. DH Shelling, 1935¹.

Despite significant literature on the effects of parathyroid hormone (PTH) in humans, dogs, sheep, rabbits, rats and mice, and the abundant *in vitro* studies of bone and osteo-sarcoma cells, there has been little advance in our understanding of how the variables summarized by Dr Shelling in a 1935 review contribute to PTH mechanisms of action in bone. *In vivo*, dosing regimens, duration of treatment and the dose magnitude determine if the outcome is anabolic, in which there is a gain in bone mass due to pronounced stimulation of bone formation, or catabolic, in which stimulation of resorption, combined with a reduction in bone surfaces available for formation, result in a net loss of bone mass over time²⁻⁶. Between these two extremes,

PTH may stimulate activation frequency to accelerate bone turnover, but, because there is coordinated up-regulation of both formation and resorption, bone mass itself remains unchanged. Even though bone mass is unchanged, bone is redistributed and architecture may show dramatic changes under conditions of increased turnover. This review will provide a brief historical perspective on work testing the hypothesis that PTH induced an anabolic effect in bone, and then focus on studies of large animal models, and selected studies of cell and molecular changes activated during the induction phase, to gain insights into what is known of the hormone’s anabolic actions on the skeleton.

The histological studies early in the last century on rats, rabbits, cats and guinea pigs using a crude extract of parathyroid hormone, PTE, showed a dramatic increase in bone following an episode of marked resorption and inflammation⁷⁻¹¹. It was not clear what actions were due to PTE and what to the contaminants in the extract, but this early work clearly associated an increase in osteoblast number and bone formation as a key outcome of exposure to pharmacological parathyroid hormone⁷⁻¹¹. Interestingly, the

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original patent litigation on claims for PTE ignored this anabolic action of parathyroid hormone, and raged on the issues of who first used acid extraction to isolate the active moiety from bovine parathyroid glands, and who first demonstrated that the active extract from the parathyroid glands raised serum calcium. Work on Hansen's extract supported the former claim, and work on Collip's extract supported the latter claim¹². Collip's extract was eventually marketed as parathormone to treat hypocalcemia and tetany, and to provide replacement therapy for patients with hypoparathyroidism¹³. For the next 40 years, the ability of PTE to regulate serum calcium and stimulate bone resorption in organ culture dominated the literature. Parathormone was used in nearly all research studies until the synthesis of hPTH 1-34 in the early 1970s^{5,14}. Now, nearly 80 years after the introduction of Collip's extract, it has been demonstrated that shorter sequences of PTH can be constructed to retain the ability to stimulate bone formation and increase bone mass *in vivo*^{6,15-18}, while the use of mutant PTH1 receptors show that receptor conformations may permit discrimination between ligands and subcellular sequelae¹⁹. As analogs of PTH are further refined to allow us to discriminate between different actions of PTH and as we improve our understanding of the consequences of activating the PTH1 receptor, it is possible that, as in the 1920s, the mechanisms uncovered may lead to additional applications of PTH to human conditions.

In careful descriptive work that is seldom cited now, once daily injections of PTE stimulated osteoblasts and increased bone mass in thyroparathyroidectomized rats lacking endogenous hormone and in hypophysectomized rats given concurrent growth hormone^{20,21}. In mice, PTE increased bone formation and bone mass²². Many of these studies have since been repeated with hPTH 1-34 and the results confirmed (see review²³, update 2001 in press)²⁴. The younger the age at which treatment was started, the more exaggerated the effect of PTE²¹. The requirement for growth hormone (GH) suggested by the studies on hypophysectomized rats, may change once peak skeletal mass has been achieved. Growth hormone, or IGF-I dependent-GH is required for the anabolic effect of PTH during the "adolescence" phase of skeletal growth, but may not be necessary after skeletal maturation in this species; GH has minimal to no effect when given exogenously to PTH-treated mature rats²⁴⁻²⁸. As PTH regulates calcium homeostasis, calcium itself may play a role in the anabolic mechanisms. Increasing calcium in the diet from 1.7% to 8.5% induced hypercalcemia and exacerbated the "osteosclerosis" induced by once daily PTE²¹. Most rodent diets contain approximately 1.1% calcium, which, given the requirement in these species for 0.5%, may be sufficient to mask some of the effects of PTH, whether given in anabolic or catabolic regimens. As calcium is regulated by the vitamin D metabolite, 1,25-dihydroxyvitamin D 1,25(OH)₂D₃ and as PTH raises serum 1,25(OH)₂D₃ *in vivo* and interacts with 1,25(OH)₂D₃ in several molecular pathways in cultured bone cells, irrespective of regimen, 1,25(OH)₂D₃ might mediate or contribute to PTH mechanisms

of action on bone *in vivo*. However, experiments in rats have failed to show that 1,25(OH)₂D₃ contributes to the anabolic skeletal effects of PTH²⁹⁻³². The increase in serum 1,25(OH)₂D₃ is likely more relevant to increased calcium absorption by the gut^{33,34}, and the improvement in total bone mineral content of the skeleton in both rats and humans³⁵⁻³⁸.

When hPTH 1-34 became available in the 1970s, studies on a limited number of retired racing greyhounds, presumed to be an osteopenic model, showed PTH induced a net gain in calcium accretion and activated bone resorption^{5,39}. Small clinical trials were conducted in the 1970s to determine if PTH could be used as an anabolic therapy for osteoporosis⁴⁰⁻⁴³. Although the balance of the evidence suggested anabolic gains in bone mass, there was considerable variability in the data due to the small number of subjects. The outcome in terms of fracture reduction was uncertain. Most recently, a large clinical trial of postmenopausal women with osteoporosis showed PTH dose-dependently increased spinal bone mass by 12-15% and femoral bone mass by 3-6%^{36,44}. This anabolic gain in bone was associated with a >65% reduction in vertebral fractures and a 54% reduction in non-vertebral fractures. With this knowledge of the reduction in fractures as the final outcome in PTH-treated humans, some of the perplexing questions on the actions of PTH on the skeleton can now be addressed by published animal data.

Overview of preclinical studies

During the past decade, most *in vivo* studies of PTH pharmacological actions used rats, and more recently, mice. The skeleton of rats has proved useful to understand alterations in modeling drift and bone growth processes, and as a general predictor of the effect of pharmacological agents on bone mass in humans. Enhancement of formation surface by PTH-stimulated endosteal bone surface apposition may relocate bone in space and change its shape to maintain or increase bone strength and bone mass in all species. The frequent use of ovariectomized rats to confirm the anabolic actions of PTH, although necessary for the preclinical development of a potential treatment for osteoporosis, has confounded interpretation of PTH mechanisms of action *in vivo*, as neither the mechanisms underlying the skeletal response to ovariectomy nor the superimposed effects of PTH are well understood. Studies to determine if the genetic pathways activated by PTH in ovariectomy are equivalent to those activated by PTH in intact rats are rare. *In vitro*, there may be some suppression of genes and signal transduction regulated by PTH in the presence of estrogen and androgen^{2,45}. Despite this caveat, hPTH 1-34 increased bone mass equivalently in ovariectomized and orchidectomized young rats⁴⁶. Suggesting a possible age-dependence in PTH responsiveness, perfused long bones of puppies generated more cAMP than did bones of adult dogs⁴⁷. Whether this difference in cAMP resulted in a similar difference in bone measures was not explored. *In vivo*, there were no significant biological differences in the effects on bone between hPTH

1-34 and the full-length recombinant hormone, hPTH 1-84⁴⁸⁻⁵⁰. In mature ovariectomized rats, treatment with PTH or one of its analogs increased bone mineral density (BMD) by more than 2-4-fold in the spine and long bones after 6 months⁵¹⁻⁵³. In osteonal skeletons, PTH-induced bone mass gain, based on DXA measures, was in the range of 8-15% in the spine of ovariectomized monkeys after 18 months^{35,54}, and bone strength increased proportionately (Fig. 1).

PTH regulation of the balance between trabecular and cortical bone

Observations of the skeletons of hyperparathyroid patients suggested that excess PTH shifts the balance of bone mass from trabecular to cortical compartments of the skeleton⁵⁵⁻⁵⁸. Clinical trials of PTH in osteoporotic humans have shown that therapeutic use of PTH strengthens bones, and increases bone mass at trabecular bone rich sites with little or no effect on bone mass of cortical bone-rich sites^{2,36,44,59-63}. In one of the first demonstrations that cortical bone was not lost at the expense of trabecular bone gain, a study of young rats showed a dose-dependent increase in both compartments⁶⁴. In rats, the continued apposition of bone on cortical endosteal and periosteal surfaces permits formation to outpace resorption to increase cortical thickness and area^{50,51,66-72}. A large number of rat studies have repeatedly shown that PTH 1-34, PTH 1-84, PTHrP 1-36, PTHrP 1-34 and analogs of PTH and PTHrP, increase trabecular and cortical bone mass by stimulating bone formation, to increase the resistance of bones to fracture at all sites tested^{2,3,5,44,65}.

Because cortical bone structure in rats and mice differs compared to humans and larger animals with osteonal bone, the clinical concerns of the cortical bone response cannot be

entirely addressed using rat and mouse models. It remains to be seen if this difference in cortical bone response to PTH is due to differences in geometry and structure, or if there are PTH-driven species-specific molecular mechanisms. Despite an increase in cortical bone formation rate, beagle dogs, treated with intermittent PTH, failed to show any increment in bone mass, porosity or cortical thickness^{73,74}. Because of the sensitivity of dog kidneys to PTH and their susceptibility to PTH-induced hypercalcemia, it is very difficult to reach a bone-effective normocalcemic dose in dogs. In one long-term study, OVX monkeys were treated with hPTH 1-34 at 1 $\mu\text{g}/\text{kg}$ or 5 $\mu\text{g}/\text{kg}$ once daily for 18 months, or once daily for 12 months, followed by withdrawal of PTH. Whole body bone mineral content (WBBMC) of PTH-treated monkeys was increased compared to controls^{35,54} (Fig. 1). This gain was evident within the first 3-6 months of treatment^{35,66,75}. Analysis of specific bones confirmed an increase in bone mass at the spine and in bone volume at the femoral neck^{35,54,76}. Increased bone mass was associated with improved biomechanical properties as the yield strength of the lumbar vertebrae increased by >40%, and that of the femoral neck by up to 23% in a dose-dependent manner (Figs 1,2)^{4,77,78}. Histomorphometry confirmed that the gain in bone mass was due to increased bone surface apposition^{66,76,79}. PTH-treated monkeys exhibited significant remodeling of their trabeculae, such that trabecular number and connectivity increased as the bone formation rate increased^{3,66,76} (Fig. 3).

Using observations of hyperparathyroid patients as a precedent (review⁸⁰⁻⁸²), there was concern that, by increasing cortical porosity, PTH might increase the risk of fracture in osteoporotic patients. Studies of intact rabbits and the OVX monkey study have shown complex responses in cortical osteonal bone during PTH treatment^{70,83,84}. In cortical and trabecular bone, PTH did not further accelerate the increase

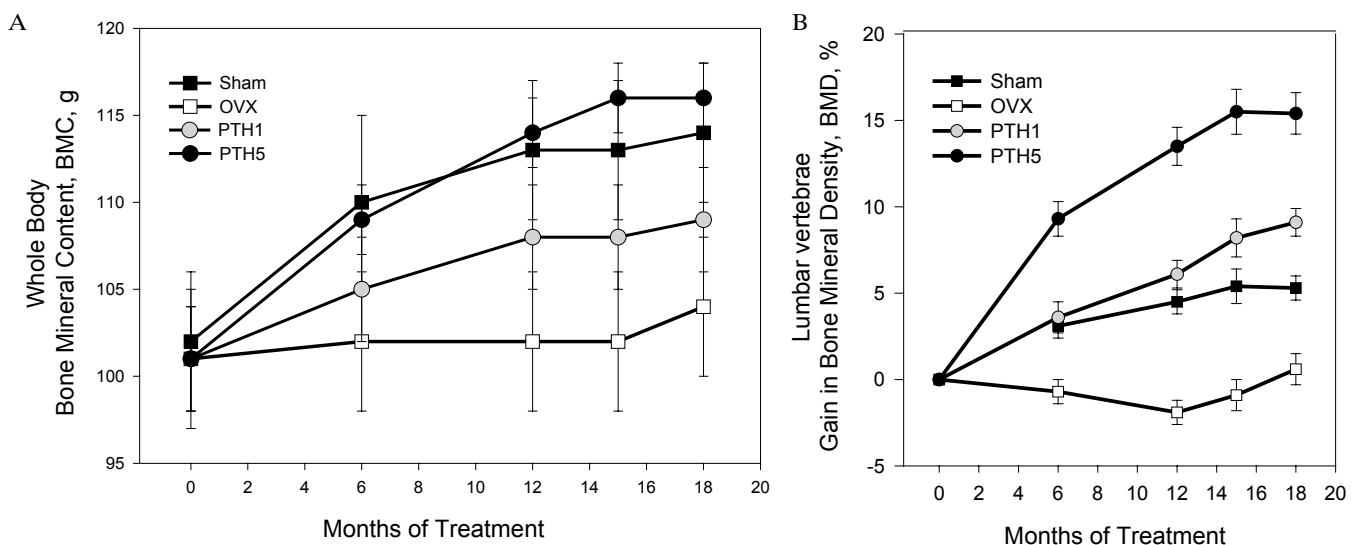


Figure 1. Changes in (A) whole body bone mineral content (BMC) and (B) lumbar vertebral bone mineral density (BMD) in control sham (Sham) and ovariectomized (OVX) monkeys given vehicle, compared to ovariectomized monkeys given rhPTH 1-34 at 1 $\mu\text{g}/\text{kg}$ (PTH1) or 5 $\mu\text{g}/\text{kg}$ (PTH5), once daily for 18 months. The change is expressed as percent increase over baseline values at time 0. Note the increment gained with PTH occurs early in treatment and is greater than either sham or ovariectomized controls^{35,54}.

in activation frequency, a measure of bone turnover, induced by OVX alone^{70,76,79}, and the increase in trabecular bone was not at the expense of cortical bone^{35,67}. There was significant stimulation of endocortical bone formation while periosteal formation remained equivalent or slightly higher than controls^{70,83,84}. Unique to osteonal bone, there was an increase in newly forming osteons and an increase in porosity of intracortical bone (Fig. 4)^{70,79,83-85}. The presence of porosity, as a reflection of remodeling space, was dose-dependent^{79,83,85}. No differences in cortical thickness or porosity were found in either dogs or OVX monkeys at sites of mixed trabecular and cortical bone, such as the vertebral shell^{66,73,74,76}. Analyses of the localization of the porosities to endocortical, intracortical and periosteal zones of the midshaft of long bones, showed that they occurred predominantly in the endocortical zone^{79,83-85} (Fig. 4). As this zone is closest to the neutral axis and buttressed by the increase in endocortical bone (Fig. 5), the cross-sectional moment of inertia, stiffness and ultimate force characteristics of strength remained stable^{79,83-85}. Unlike pathological conditions such as hyperparathyroidism in humans, intermittently administered pharmacological PTH has anabolic effects in both trabecular and cortical bone. Bone apposition is promoted on trabecular and cortical endosteal envelopes and the cortical periosteal envelope, while intracortical bone is renewed through remodeling. Taken together, these data suggest the concept that biomechanical properties of bone are maintained or enhanced as a consequence of PTH effects on cortical bone, so that susceptibility of bone to fracture remains unaffected or less likely.

Early effects of PTH on cortical thickness

A clinical concern remains that, if the increase in porosity due to increased bone turnover, occurs prior to deposition of

PTH-induced new bone formation, an osteoporotic patient with thinned cortical bone may be susceptible to fracture early in treatment. A large clinical trial showed that there was a significant reduction in non-vertebral fractures, and the earlier smaller clinical studies did not find increased susceptibility to fracture at cortical bone sites^{2,36,59,60}. As the criteria for patient selection in clinical trials rests on estimates of bone mass or spine fractures, rather than on risk of fractures at sites where cortical bone predominates, the question of whether PTH may initiate resorption prior to formation in cortical bone, remains to be answered. In rats, inhibition of resorption by calcitonin or a bisphosphonate prior to starting PTH did not abrogate its anabolic effect in intact rats⁸⁶. Bones of young microphthalmic and osteosclerotic mice with impairment or absence of resorption exhibited the anabolic effect of PTH²². Most recently, the time sequence of events has been studied in cortical bone of intact rabbits treated with hPTH 1-34 during the 1st remodeling period, calculated as 70 days^{84,85}. The percent fluorochrome-labeled (new) osteons and endocortical bone formation increased within 35 days to enhance biomechanical properties of bone, while the increase in porosity was not significant until 70 days⁸⁴. In human studies (which did not include placebo controls), the initial biochemical and histomorphometric changes were consistent with an immediate stimulation of bone formation and a later increase in resorption⁸⁷⁻⁸⁹. Collectively, these data suggest that PTH “braces” the bone by immediately stimulating formation at both modeling and remodeling sites, so that an initial period of increased susceptibility to fracture would not be predicted. While these studies provide useful insights, more data are needed on a model for the osteoporosis case in which cortical bone is precariously thin prior to initiating PTH treatment, to fully assuage the clinical concerns.

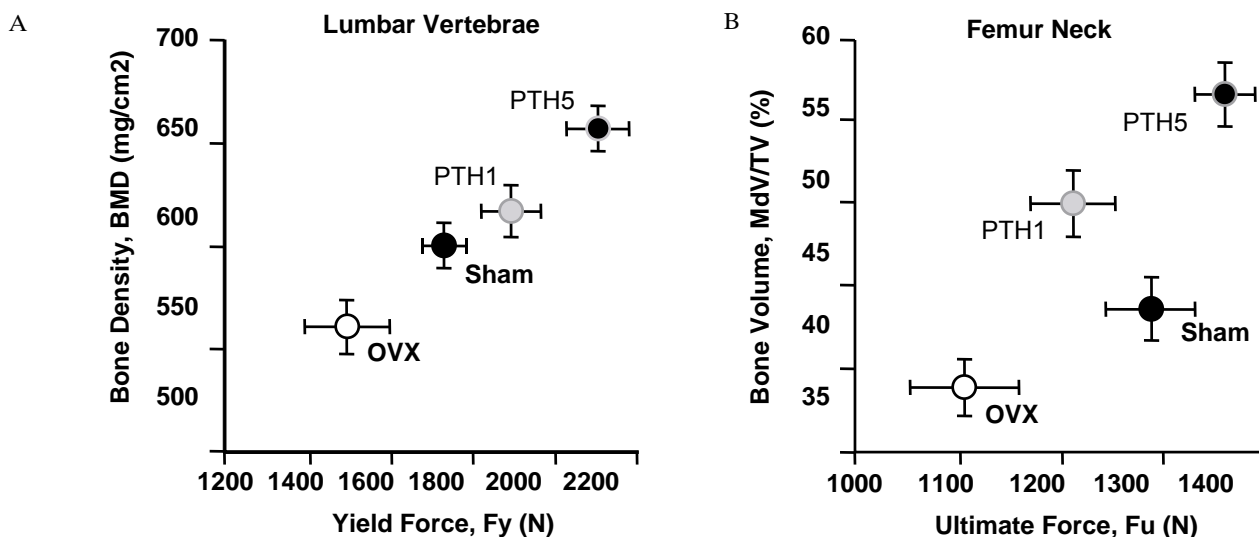


Figure 2. The gain in bone mass and strength in control sham (Sham) and ovariectomized (OVX) monkeys given vehicle, compared to ovariectomized monkeys given rhPTH 1-34 at 1 µg/kg (PTH1) or 5 µg/kg (PTH5), once daily for 18 months. A: vertebral mass as BMD and resistance to fracture as yield force. B: femur neck mass as percentage trabecular bone volume (BV/TV) plotted against strength expressed as ultimate force. The values for vertebrae are significantly different from each other. In the femur neck, PTH treated monkeys were significantly different from OVX control, and PTH5 was statistically significant compared to sham controls^{35,77,78}.

Time-dependence of the cell and molecular effects during induction of PTH response

The *in vivo* time-dependence of PTH effects in bone and their correlation with transient changes in serum calcium are seldom considered. An often forgotten study, in which young rabbits were given PTE and events tracked over time in cross-sections of cortical bone autoradiographs of very young rabbits, showed that there was an initial decrease in RNA synthesis, followed by a marked increase at 18-24h⁹⁰. The increase was associated with up-regulation of osteoblasts and their progenitors, a decrease in endosteal osteoclasts and an increase in intracortical osteoclasts⁹⁰. The histological events in bone were correlated with the transient changes in serum calcium, suggesting a possible role for calcium fluxes immediately following PTH injection^{5,90}. In rats, increases in bone matrix proteins and bone-forming surfaces in trabecular bone occur within 1-24h of the first injection of hPTH 1-34, while resorption measures remain unchanged⁹¹⁻⁹⁷. It may be that each envelope of bone has a unique response to PTH, as suggested by recent work with mice⁹⁸⁻¹⁰⁰. The periosteal surfaces may be especially sensitive to mechanical loading as a rat study showed synergistic anabolic effects of PTH and mechanical loading on periosteal bone formation¹⁰¹. Perhaps, the down regulation of genes and proteins should be examined with the same enthusiasm as up-regulated genes in our attempts to probe molecular mechanisms.

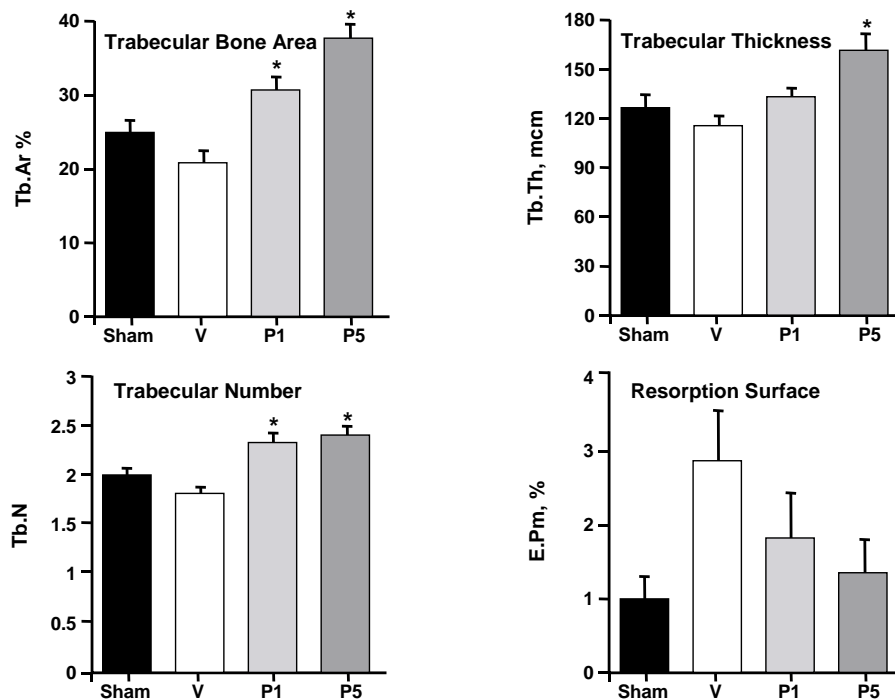


Figure 3. Bone histomorphometry of trabecular bone in the iliac crest of sham control (Sham) and ovariectomized monkeys after 6 months of treatment with vehicle (V) or rhPTH 1-34 at 1 µg/kg (P1) or 5 µg/kg (P5). Note that trabecular area and number are increased at both doses of PTH, consistent with improved connectivity, but significant differences in trabecular thickness were observed only at the higher dose⁷⁶. Statistical significance compared to OVX controls, $p < 0.05$ *.

Regulation of immediate early genes by PTH in rats and mice

During induction of the anabolic response, PTH regulates many osteoblastic genes, mainly through the cAMP-PKA signaling pathway, with the protein kinase C pathway probably also contributing to signal transduction^{6,102,103}. Following once daily injection for 1-5 days, PTH-stimulated proteins include transcription factors, matrix proteins required for new bone formation, proteins associated with matrix degradation and turnover, and osteoclast differentiation proteins. Studies in young mice⁴⁸ and rats¹⁰⁴⁻¹⁰⁶ indicate that PTH up-regulates differentiation in metaphyseal trabecular bone cells in a dose-dependent manner, and this has been linked to prior transient stimulation of the transcription factors *c-fos*, *c-jun*, and *c-myc* within 30 minutes. As expression of these genes can be linked to proliferation, differentiation, function and apoptosis, a selective link to osteoprogenitor differentiation remains speculative at this time. Of the early response genes examined, *c-fos* shows the greatest magnitude of change in response to PTH⁴⁸. Its up-regulation is not dependent on PTH regimen. *In vitro*, exposure to PTH (1-34) and PTH-related peptide (PTHrP [1-34]) also rapidly induced *c-fos* gene expression in bone cell lines¹⁰⁶⁻¹⁰⁸ in a proliferation and differentiation-dependent manner¹⁰⁹. Demonstrating that up-regulation of one gene occurred in different cell types

over time, up-regulation of *c-fos* occurred initially in osteoblasts, and subsequently in stromal cells and osteoclasts in rats treated with a single injection of PTH¹¹⁰. The implications of time-dependent changes initiated by PTH *in vivo* have not been well appreciated at the cell and molecular level, or by *in vitro* models.

The histological and quantitative bone data showing changes in bone mass and shape after several remodeling cycles suggest additional mechanisms may operate after prolonged PTH treatment, at least in rats. As osteosarcoma was associated with lifetime treatment of young growing rats with PTH⁴⁴, mechanistic studies are needed of the effects of prolonged PTH treatment. As 0.1-6.0% of laboratory rats and mice exhibit spontaneous osteosarcoma towards the end of their life^{111,112}, near-lifetime exposure to PTH in these species may promote, but not induce, cell transformation. The relevance of this phenomenon to the osteonal skeleton of larger mammals and humans remains uncertain.

There have been no long-term studies of animals, in which the response to PTH is independent of the growth and developmental skeletal processes present in rats and mice.

In young rats, other genes upregulated within 1h of PTH injection, include immediate early genes, IL-6 and LIF and RANKL, RGS2, ADAMS-T and matrix metalloproteinases such as collagenase 9 (gelatinase B), while expression of the PTH receptor, histone H4 and OPG are decreased^{92,95,97}. Interestingly, effects on mRNA expression of osteocalcin, growth factors such as IGF-I, FGF and TGF β , and their receptors, are not detectable until days or weeks after initiation of treatment^{91,113-116}. This delay in up-regulation suggests that an increase in growth factors and osteoblast-specific factors may be more an indication of the highly significant increase in osteoblast numbers and function, than evidence for a role as primary mediators of the anabolic actions of PTH. The specificity of the regulated genes identified to date in anabolic protocols of PTH is unknown, as regulated genes associated with continuous infusion or with animal models of hyperparathyroidism have still to be identified. In contrast to intermittent *in vivo* exposure, PTH, *in vitro*, acts on differentiated osteoblasts to inhibit expression and synthesis of matrix proteins, including collagen 1, osteocalcin and alkaline phosphatase, independently of exposure duration¹¹⁷⁻¹¹⁹. Following continuous infusion of PTH in adult rats, peritrabecular fibrosis and focal resorption were observed, together with hypercalcemia and increased calcitonin^{48,120-122}. The fibrosis subsequent to PTH regulation of collagen synthesis may be mediated by a changing ratio between IGF-I and IGF-binding proteins^{123,124}, suggesting an unconventional role for the IGF-axis. These shifts in distribution and magnitude of IGF-1 and IGF-

binding proteins may regulate cell fate determination of the multi-potential progenitors in close proximity to active osteoblasts. *In vitro* studies, using bone organ culture or cells isolated from fetal or neonatal animals, suggest that IGF-I may be one of the mediators of PTH effects on skeletal growth and maturation processes via its stimulatory effects on osteoblasts¹²⁵. Alternatively, as IGF-I inhibits collagenase¹²⁶, IGF-I may mediate a different aspect of the anabolic mechanism, namely regulating the process by which osteoblasts condition the bone surface as a prerequisite to attract osteoclast progenitors to bone. As skeletal cells secrete the six known IGF binding proteins (IGFBPs) and 2 of the 4 known IGFBP-related proteins, there may be additional levels of regulation if IGF-I mediates actions of PTH *in vivo*^{123,124,127,128}. As microarrays add to our knowledge of changing patterns of gene expression over time and with different PTH regimens, we will better understand the significance of time-dependent changes representing multiple regulatory pathways.

PTH-activation of genes and proteins associated with bone turnover

Cell outcomes, which regulate bone balance via bone turnover, appear to depend on frequency and duration of exposure to PTH^{2,30,122,129-131}. In rats, upregulated expression of both matrix degrading proteins, such as matrix metalloproteinases (MMPs) and ADAMTS-1 (A Disintegrin And Metalloprotease with ThromboSpondin motifs) and cytokines associated with regulating matrix degradation and turnover, such as interleukin-6 (IL-6), IL-11 and osteopontin, has been a consistent finding^{92-94,97,132,133}. Actions of matrix degrading enzymes to recondition the bone surface may lead

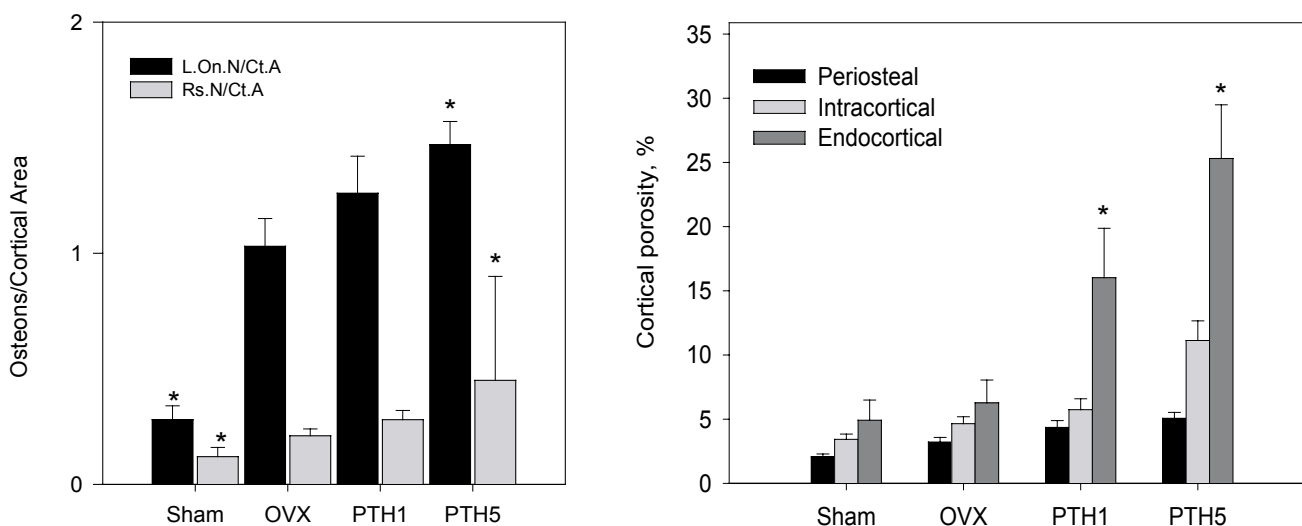


Figure 4. Increased remodeling in the humerus midshaft of ovariectomized monkeys given rhPTH 1-34 at 1 μ g/kg (PTH1) or 5 μ g/kg (PTH5), once daily for 18 months, is associated with new osteon formation. Bone formation in osteons (L.On.N/Ct.A) predominates over new osteons initiated by resorption (Rs.N/Ct.A). Cortical porosity is increased in ovariectomized controls (OVX) compared to shams (Sham). Cortical porosity in PTH-treated monkeys is located predominantly within the endocortical region. The dose-dependent effect of PTH-induced porosity on biomechanical properties of the midshaft is minimal, as the endocortical region is close to the neutral axis of the cross-sectional moment of inertia. Statistical significance compared to OVX controls, $p < 0.05$ *⁷⁹.

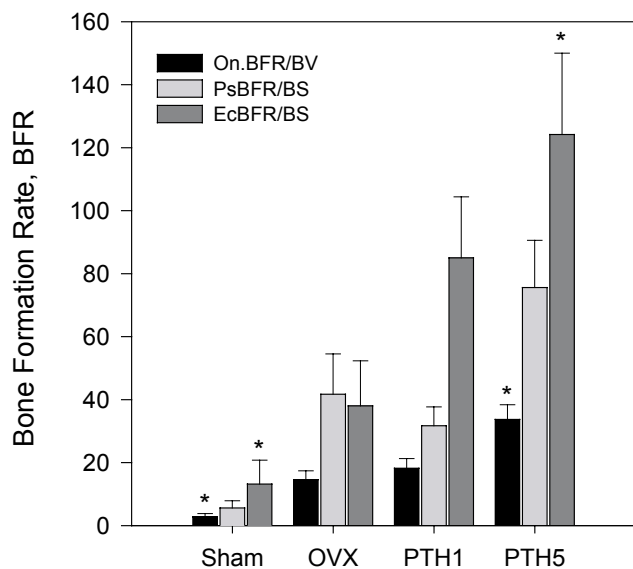


Figure 5. Bone formation rates in the periosteal, intracortical and endocortical regions of the femur mid-shaft of sham (Sham) and ovariectomized (OVX) controls compared to ovariectomized monkeys given rhPTH 1-34 at 1 $\mu\text{g}/\text{kg}$ (PTH1) or 5 $\mu\text{g}/\text{kg}$ (PTH5), once daily for 18 months. Ovariectomy increases the bone formation rate in periosteal (Ps.BFR/BS) and intracortical (On.BFR/BV) regions. The anabolic effects of PTH increase bone formation in the endocortical (Ec.BFR/BS) and intracortical regions⁷⁹. Statistical significance compared to OVX controls, $p < 0.05$ *.

to osteoblast detachment resulting in osteoblast apoptosis. A transient increase in apoptosis in proliferating cells and osteocytes of young rat metaphyses was observed during the initial response to PTH¹³⁴. Activation of matrix degrading enzymes and associated phenomena may result in reconditioned bone surfaces, which can serve as an attractant for either newly differentiating osteoblasts to increase bone-forming surfaces (anabolic action), or for differentiating osteoclasts to continue resorption of old surfaces (catabolic action)¹³⁷. While the rat work strongly implicates matrix degradation as an initial event in the anabolic PTH response, the cytokines and patterning genes required to regulate PTH-induced restructuring and remodeling of bone in animal models in which osteonal bone structure and remodeling processes predominate, have not been investigated.

As discussed earlier, resorption is an integral component of the stimulatory effect of PTH in bone turnover^{138,139}. Evidence to date suggests its activation is delayed, enabling accrual of bone by osteoblast action first^{84,87,88}. Stromal cells and osteoblast lineage cells regulate osteoclast differentiation, by controlling synthesis of OPG and RANKL, the ligand for the osteoclast progenitor receptor, RANK^{138,139}. These two secreted proteins compete for binding to RANK, a TNF receptor family member¹³⁹. If RANKL binding to RANK predominates, as seen following PTH treatment of cultured osteoblast-like osteosarcoma cells transfected with the PTH1 receptor¹⁴⁰ or in bone organ culture, osteoclast progenitors differentiate into osteoclasts^{105,139}. In addition, studies in a

variety of bone cell lines indicate that PTH down regulates OPG, a potent inhibitor of osteoclast formation and function^{105,138}. In young rats, mRNA expression for RANKL increases while that for OPG decreases within 1h of injection⁹⁵. Although activation of osteoclasts by PTH may be a direct effect due to shifts in the ratio of OPG and RANKL expression in osteoblasts and stromal cells, the shift in matrix protein synthesis by osteoblasts to a more fibroblast-like profile^{122,141,142} may provide an extracellular matrix feedback signal to activate increased bone turnover. One limitation of all these studies has been our lack of knowledge of how increased activation frequency may regulate genetic or cell events to favor either formation (anabolic effect of intermittent PTH) or resorption (catabolic effect of continuous PTH). A mathematical model that assumes a longer delay in osteoclast activation (due to a requirement for signals from the osteoblast to osteoclast progenitors) than the delay required for osteoblast differentiation, argues that osteoblast function will predominate with intermittent PTH, while resorption will be greater with continuous PTH¹⁴³. This speculation is supported by preliminary unpublished data showing that the *ex vivo* induction of osteoclasts was delayed in mice given once daily injections of PTH until 28-31 days; under conditions of continuous infusion, increased *ex vivo* induction of osteoclasts was detected within 14 days¹⁴⁴.

Effects of PTH on osteoblast cell fate

Research of the past decade has reproducibly shown the osteoblast and its progenitor to be the primary target of PTH *in vivo*. The mechanisms of action are still not well understood, although we know that PTH regulates gene expression in osteoblasts supporting synthesis of various proteins involved in bone formation and resorption. While *in vitro* studies have shown inconsistent effects of PTH on bone cell proliferation^{145,146}, studies in both young and old rats have provided no evidence to support PTH as a stimulator of osteoblast proliferation¹⁴⁷⁻¹⁴⁹. In young rats, there are large pools of proliferating cells underlying the growth plates, the metaphyseal cortical endosteal surface and the diaphyseal periosteal surfaces^{150,151}, which may be regulated by PTH^{91,152-154}. The absence of evidence to support an initial stimulation of proliferation, combined with the presence of large numbers of proliferating cells *in vivo*, and the fact that these cells can be enabled to differentiate into different lineage cells, suggest that PTH may recruit proliferating cells into the osteoblast differentiation pathway to increase the number of osteoblasts^{91,152-154}. In older animals with closed growth plates and few proliferating progenitors¹⁵⁵, it has been speculated that PTH and its analogs increase osteoblast number by stimulating differentiation of quiescent bone surface cells^{2,59,66,75,89,147,156}. PTH may also increase the number of bone cells by inhibition of cell death (apoptosis) in existing osteoblasts^{134,157-159}. It is not clear if osteoblasts and osteocytes remain fully functional if apoptosis is inhibited or delayed. The inhibitory effect on osteoblast apoptosis may be a direct

effect of PTH, or an indirect effect associated with acceleration in bone turnover. The long-term consequences of PTH treatment on bone cell proliferation and apoptosis after multiple bone turnover cycles associated with the PTH-induced increase in activation frequency, have not been studied.

Reversal of effects upon withdrawal of PTH

Long-standing, unanswered clinical questions relate to the consequences of stopping PTH treatment, the duration of the benefits gained, and if additional agents will be needed to retain PTH-induced new bone. A small study of osteoporotic humans, given PTH for 1 year followed by a one-year course of the bisphosphonate, alendronate, showed continued increase in bone mineral density in the year following PTH withdrawal, but the effect on fracture reduction could not be assessed in the small sample¹⁶⁰. It remains to be determined whether post-PTH gain in BMD was due to the anti-resorptive effects of alendronate alone, to an effect on resorption occurring as a consequence of PTH withdrawal, or to a complementary effect to increase mineral content of newly forming osteons induced by PTH. With few exceptions, studies of withdrawal have been in humans treated with an anti-resorptive or HRT alone or in combination with PTH¹⁶⁰⁻¹⁶² so that the consequences of PTH alone cannot be judged. Bone outcomes following ablation surgery for treatment of long-standing hyperparathyroidism^{56,57,163,164} suggest that bone mass may continue to increase. Data that evaluate consequences of PTH withdrawal are available from animal studies, which include intact and ovariectomized rats^{65,165-170}, dogs⁷⁴ and ovariectomized monkeys^{54,76-79}.

In young male rats, withdrawal of exogenous PTH resulted in loss of the anabolic effect on bone formation within 24-28h, and a reversal to control measures of bone mass within 12 days¹⁷¹. Treatment of mature ovariectomized rats or dogs with hPTH 1-34 or hPTH 1-84, followed by an equivalent or longer period of withdrawal of treatment, showed the anabolic gains in bone mass and strength were reversed within this time frame^{65,74,165-167,169}. Treatment during the withdrawal period with an anti-resorptive slowed the loss of PTH-induced bone gain in rats^{65,165-168}. Collectively, rat studies suggest that the speed of the withdrawal response may be dependent on the age of the rat and the duration of treatment. In larger animal models with osteonal bone, the loss of PTH skeletal benefits seems to be slowed for at least 2 remodeling periods, due to a decrease in remodeling transients and the associated maturation and mineralization of newly formed osteons^{76,79}. An unexpected finding was the favorable shift in mineral distribution and size in pQCT images that correlated with the retention of improved biomechanical properties of bone after withdrawal of PTH treatment^{57,77,78}. The delayed effects occurring as accelerated osteonal formation returns to control levels may mask loss in bone mass from endosteal surfaces, but appears to be sufficient to sustain the gains in strength over 2 remodeling periods, at a level

significantly above that of ovariectomized controls^{54,77,78}. The extent to which these phenomena mimic those elicited in osteoporotic humans has still to be ascertained. Given the validity of much of the preclinical data to predict events in osteoporotic humans to date, it is likely that there will be a period of at least two turnover cycles in which the benefits of PTH may continue to accrue in its absence.

Conclusions

The anabolic effect of PTH on the skeleton of most vertebrates, including humans, is undisputed after several decades of research with parathyroid hormone extract, and more recently, with the full length hormone, hPTH 1-84, and a variety of analogs, including hPTH 1-34. A large clinical trial showed that the anabolic effect to increase bone mass was associated with a decrease in new fractures to a greater extent than has been reported with anti-resorptives. Fears that cortical bone might increase in porosity and decrease in mass have not been substantiated in experimental animal models. PTH appears to buttress cortical bone by increasing appositional bone formation to increase cortical bone width, thus offsetting the later increase in porosity. The distribution of porosities and remodeling intracortical sites is predominantly in the endocortical bone, so that there is little effect on biomechanical measures such as cross-sectional moment of inertia. PTH activates many of the classic early immediate genes, but the sequelae and selectivity of such activation is not known. Mechanisms that differentiate between the bone loss associated with pharmacologic PTH infusion, versus the bone gain following intermittent pharmacologic PTH, are still not understood. Withdrawal of PTH correlates with reversal of the effects on bone turnover, but reversal of skeletal benefits does not occur for at least two remodeling periods. Rather like the authoress of the stories in the classic "The Arabian Nights", PTH continues to sustain the mysteries around its mechanisms of action a century after its discovery.

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