DOES PTH STIMULATE BONE FORMATION?

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Internal Medicine Grand Rounds

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I. Concept of Fracture Threshold and Importance of Agents That Can Stimulate Bone Formation

Fig. 1 Therapeutic Implications of Fracture Threshold

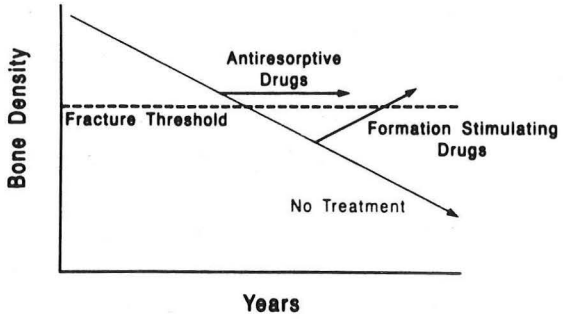


Fig 1 Therapeutic implications of fracture threshold

If a patient with decreasing bone density is seen at a time before density has fallen below the fracture threshold, then anti-resorptive agents, such as estrogen, calcitonin or bisphosphonates may be given to prevent development of osteoporosis.[1] These agents primarily stabilize bone mass and prevent further loss of bone, although a transient small increment in mass is often reported, particularly in patients with elevated levels of bone remodeling. This increase is not a true anabolic effect, but is related to the temporal effects on turnover in which resorption declines initially followed by a reduction in formation that may take several months.

If an individual is first seen when the bone density has already fallen well below the fractured threshold, anti-resorptive therapy may not be sufficient to prevent further fractures. Agents that could truly stimulate bone formation would be required. Although no such agent has yet been approved by the FDA, a number of substances with the potential to stimulate bone formation are currently under active investigation.

Table 1. Agents Capable of Stimulating Bone Formation

Slow-Release Sodium Fluoride

Growth Hormone/IGF-1

PTH

II. Current Status of Research on Bone Stimulating Agents

Slow-Release Sodium Fluoride

Fluoride does increase bone mass by a true anabolic action, but there has been controversy about the quality of bone formed. Two recent controlled studies failed to find a reduction in fracture recurrence.[2,3] The preparation used also produced gastric irritation, bleeding, and a peculiar periarticular pain syndrome in the feet and legs. It is

now realized that the wrong dose (too high) of sodium fluoride was used and the wrong kind (plain sodium fluoride rather than a slow-release preparation).[4] Recently, the Dallas Mineral Metabolism Group reported a blinded interim analysis of a placebo-controlled, randomized trial of slow-release sodium fluoride and calcium citrate supplementation.[5] The absolute dose of sodium fluoride was one-third less than had been used in the previous trials and the slow-release formulation was probably slightly less well absorbed than plain sodium fluoride. As expected, vertebral bone mass increased by about 5% per year in the fluoride-treated group, but more importantly, the vertebral fracture rate decreased to about one-third that of the placebo-treated group. Bone mass in the appendicular skeleton was maintained, and no hint was apparent of an increase in appendicular fractures. Moreover, with the slow-release preparation, no difference was noted in gastro-intestinal side effects between the groups. No patient developed microfractures. The new bone formed was well mineralized under light and electron microscopy and was improved in strength or quality when tested by reflection ultrasound. Riggs and colleagues, in a reanalysis and extension of their NIH trial data, recently reported that those patients receiving a more appropriate dose of fluoride had a gain in bone mass and reduction in fractures.[6] Meanwhile, in Dallas, long-term effects are still under study. Although it is now an approved treatment in several European countries, sodium fluoride remains an investigational drug in the United States.

Growth Hormone/IGF-1

Pituitary growth hormone (GH) is a classical endocrine hormone with profound effects on somatic growth and body composition. It is now recognized that circulating concentrations of GH decline with advancing age, and that this decline is accompanied by reduced levels of insulin-like growth factors (IGF's), the putative mediators of many of the hormone's actions. Indeed, it was recently reported that there is a 60% loss of human skeletal IGF-1 between the ages of 20 to 60 years.[7] Normal human aging is associated with important alterations in body composition that are also characteristic of GH-deficient children. These include increases in adiposity and declines in muscle mass and strength, and of course, in bone mineral density. Thus, it seems reasonable to ask whether some age-related changes in body composition are brought about by a relative degree of GH deficiency, and further, whether GH or IGF-1 therapy might have clinical utility in reversing these changes. With the availability of recombinant human GH and IGF-1, therapy of adults would be a possible, if expensive, strategy.

Marcus et al. reported the effects of 7 days of rhGH to 16 healthy older men and women (>60 yr).[8] rhGH led to a brisk rise in circulating IGF-1, which was associated with striking reductions in nitrogen and sodium excretion by 38% and 50%, respectively. Urinary calcium excretion markedly increased, as did circulating osteocalcin and urinary hydroxyproline, suggesting that bone remodeling had been activated. The most widely publicized GH trial to date was reported by Rudman et al.[9] A group of elderly men with low IGF-1 levels were treated with GH (.03 mg/kg 3 times per week) over a 6 month period. Plasma IGF-1 levels rose to the youthful range and lumbar bone density increased by 1.6%, without any change in bone density at 8 other sites. Lean body mass increased by 9% and adipose tissue was decreased by 14%. The change in bone density was marginally significant at best, and the statistical analysis made no adjustment for

multiple comparisons. In another placebo controlled trial of rhGH in 22 healthy elderly women, for up to 1 year, although sustained elevations in circulating osteocalcin and bone alkaline phosphatase were observed, there were no significant changes in bone mineral density at the spine or femur.[10] GH administration is frequently associated with side effects including weight gain, fluid retention, arthralgias, decreased insulin sensitivity associated with increases in fasting blood glucose, and carpal tunnel syndrome.[10] Long-term treatment raises concern about cardiovascular and tumor risk, as may occur with acromegaly. Daily injections of rhGH for 6-12 months at doses that are close to the limit of tolerance, appear to induce a persistent increase in bone remodeling, but do not appear to produce important increases in bone mass in elderly people.

It has been postulated that IGF-1 may produce some of the anabolic effects of GH without some of the associated complications such as insulin resistance, hyperglycemia and hypertriglyceridemia. Early reports would seem to bear this out.[11] However, just as with GH, bone resorption as well as formation appears to be stimulated, and it is not yet clear whether the net balance will be positive.

IGF-1 present in the skeletal matrix may be derived from the systemic circulation or synthesized by a variety of cells present in bone. In vitro work would support an important potential role of IGF-1 in stimulating bone formation since it promotes replication and differentiation of osteoblasts, as evidenced by an increase in osteocalcin and type I collagen synthesis. In addition, IGF-1 decreases collagen degradation, suggesting a potential role in the preservation of bone matrix. Some have suggested that its release from bone matrix following osteoclastic bone resorption may be the putative "coupling factor" promoting osteoblastic bone formation.[7] A decrease in IGF-1 content in bone, as recently reported,could have important implications in the pathogenesis of osteoporosis.

It is not clear if the decrease in skeletal IGF-1 with aging is due to a deficiency in the systemic circulation of IGF-1 (both decrease by about 50% from the 3rd to 6th decades), or to impaired production of IGF-1 in the skeleton.[12] Whereas the synthesis of the systemic form of IGF-1 occurs in the liver and is GH-dependent, the synthesis of skeletal IGF-1 is modulated by different control mechanisms. In vitro studies using osteoblast cultures indicate that PTH and other agents with cAMP-inducing properties play a more significant role than GH in the stimulation of IGF-1 in bone.[13] If the decrease in the skeletal content of IGF-1 in aging is secondary to a decline in serum IGF-1 concentrations, it may be possible to restore its serum and bone levels by the administration of GH, because the levels of circulating IGF-1 are GH dependent. On the other hand, if the decrease in bone IGF-1 content is due to a decline in IGF-1 synthesis by skeletal cells, GH is less likely to be effective because skeletal IGF-1 is only modestly dependent on GH.[12,13]

The latter problem could be overcome in two ways. IGF-1 could be administered directly. However, preliminary work in humans has shown that systemic administration of IGF-1 increases both serum levels of type I procollagen propeptide (an index of bone collagen synthesis) and urinary excretion of deoxypyridiroline (an index of bone collagen breakdown).[14] These data suggested that the administration of IGF-1 increases bone

remodeling. Administration of IGF-1 has been associated with side effects including orthostatic hypotension, tachycardia, weight gain and edema.[12,14] A feared side effect of IGF-1 is the induction of hypoglycemia, because IGF-1 like insulin, increases intracellular glucose transport. However, this could be minimized by using the lowest effective doses of IGF-1. There is also the risk that chronic administration of IGF-1, like that of GH, could result in side effects analogous to the abnormalities observed in acromegaly.

Problems with the systemic use of IGF-1 could be circumvented by increasing the synthesis of the locally produced skeletal IGF-1. This has been accomplished in vitro by agents that stimulate cAMP production in osteoblast cultures, and if it were possible to duplicate this action in vivo, it could provide the basis for new therapeutic alternatives in conditions of decreased bone mass.[12,13] There is evidence that PTH, a known cAMP inducer in bone, given intermittently, increases bone formation in vitro and in vivo. The in vitro effect is due to an enhancement of IGF-1 synthesis and possibly activation or release of TGFβ. However, the mechanism of the anabolic effect of intermittent PTH in vivo may or may not involve changes in the production or activity of skeletal growth factors.

PTH

A review of investigations on the anabolic actions of PTH on bone will constitute the remainder of this Grand Rounds.

II. Lessons from Primary Hyperparathyroidism: Effects of Endogenous PTH on Bone in Humans

Hyperparathyroidism was unknown in this country until the diagnosis was first made by Eugene F. Dubois in 1926.[15] The patient was Captain Charles Martell, a mariner who had become disabled by demineralization of the skeleton during many years. Finally, a 2.5 cm mediastinal parathyroid adenoma was removed at the seventh operation in 1932. In those days, primary hyperparathyroidism (PHPT) was regarded as a rare and severe disease of bone, "osteitis fibrosa cystica."[16] The syndrome was characterized by bone pain, fractures and renal stones. The radiologically evident destructive bone lesions and histologic observations of severe unbalanced osteoclastic resorption led to the belief that high levels of endogenous PTH exerted a catabolic effect on the skeleton. Many years later, increased awareness of this disease and the availability of multichannel chemical screening dramatically changed the mode of presentation of PHPT.[17,18] Hence, for the past 15-20 years, presumably through this phenomenon of early recognition, a milder clinical phenotype is seen most often. Patients are usually asymptomatic and discovered incidentally. Hypercalcemia is mild and clinically evident bone disease is quite uncommon. In fact, recent studies of patients with mild PHPT suggest that mildly elevated circulating levels of PTH may have beneficial effects, at least on cancellous bone. This section will review these recent findings.

Although there is still some controversy in this regard,[19,20] convincing recent work from Bilezikian and co-workers at Columbia[21] has lessened concern about the development of spinal osteoporosis in patients with asymptomatic PHPT. Bilezikian's

group investigated 52 patients with PHPT.[21] They had mild hypercalcemia (Ca 11.1 ± 0.1 mg/dl) and no symptoms or specific radiologic signs of skeletal involvement. Although they were asymptomatic with respect to bone disease, 20% of the patients did give a history of nephrolithiasis. The purpose of the study was to determine whether skeletal involvement could be appreciated when more sensitive techniques such as bone densitometry and bone biopsy were utilized. A subset of this population, 31 patients, completed densitometric studies of three skeletal sites that correspond principally to cortical bone (diaphyseal radius), cancellous bone (lumbar spine) or a combination of both (femoral neck). The bone density at each site in comparison to expected values for age- and sex- and ethnic-matched normal subjects is shown in Fig. 2. At the lumbar

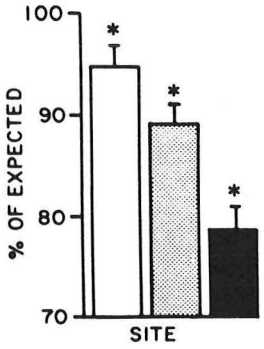


FIG. 2 Densitometric measurements at three skeletal sites (*white*, lumber; *gray*, femoral; *black*, radius) in 31 patients with PHPT who did not have any symptoms or radiologic signs of skeletal disease. The bone density at each site is shown as a percentage of values for age-, sex-, and race-matched normal subjects. (By permission from Ref. 21)

spine, the average bone mineral density was 1.07 ± 0.03 g/cm^2 which is within 5% of the expected mean for matched normal subjects. At the femoral neck, the hyperparathyroid population began to diverge from normal with a mean value of 0.78 ± 0.14 g/cm^2, 89 ± 2% of the expected value. The radius showed the greatest difference from normal. Mean bone density at this site was 0.54 ± 0.10 g/cm^2, only 79 ± 2% of the expected mean value. Bone density of the spine did not differ significantly from normal, whereas bone density of the radius showed the greatest reduction.

Twenty of the Columbia group's patients underwent histomorphometric analysis of bone obtained by transiliac biopsy.[21] Mean cortical width and cancellous bone volume are shown in Figs. 3 and 4, respectively.

Fig. 3

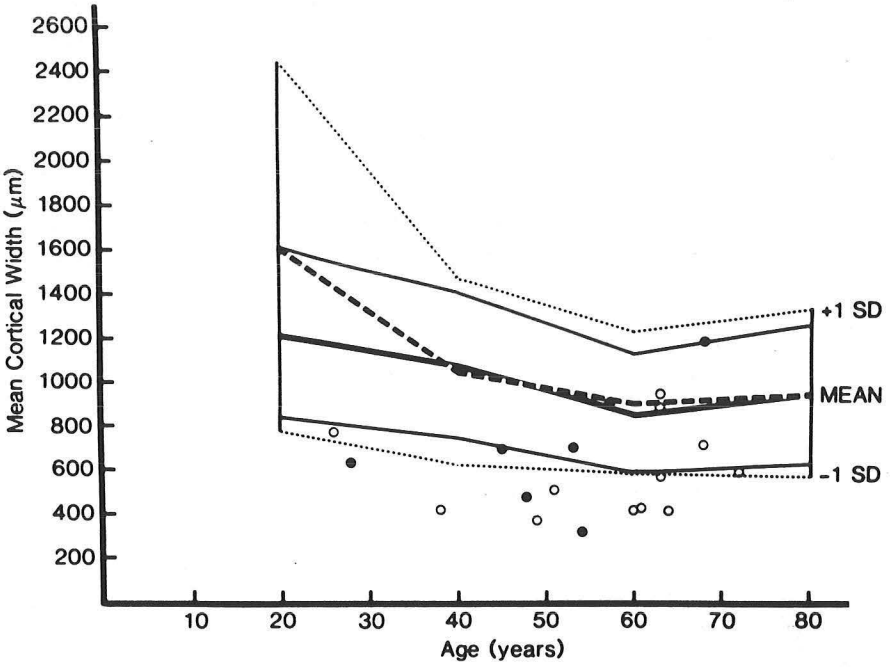


FIG. 3 Mean cortical width of bone obtained by transiliac biopsy in patients with PHPT compared to normal control subjects. Values for hyperparathyroid men are shown in *solid circles* and those for women in *open circles*. The *solid lines* depict mean ± SD values for control men and the *dashed lines* for control women. (By permission from Ref. 21)

Fig. 4

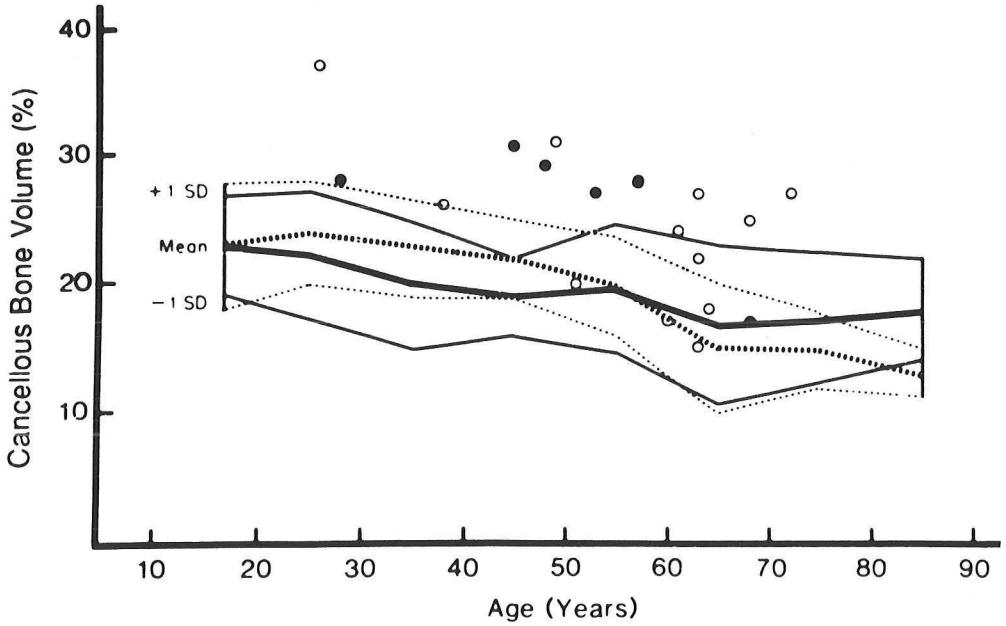


FIG. 4 Cancellous bone volume in transiliac biopsy specimens from patients with PHPT compared to normal control subjects. Values for hyperparathyroid men are shown in solid circles and those for women in open circles. The solid lines depict mean ± SD values for control men and the dashed lines for control women. (By permission from Ref. 21.)

Most patients (84%) with PHPT had cortical width below the control mean, consistent with the data obtained by single-photon absorptiometry showing decreased radial bone mineral density. In contrast to the relative decrease in mean cortical width, cancellous bone volume was preserved. Compared to control subjects, 85% of patients with PHPT had greater than average values for cancellous bone volume. Preservation of cancellous bone, as determined by histomorphometric analysis, was consistent with normal bone density of trabecular sites (lumbar spine) as determined by dual photon absorptiometry. Representative scanning electron micrographs of bone biopsy specimens from normal and hyperparathyroid subjects also showed loss of cortical bone, but preservation of cancellous bone in PHPT.[21]

Several additional histomorphometric studies have evaluated the skeletal effects of the modest elevations of PTH seen in the modern presentation of PHPT.[22-24] These studies performed on different populations over the past 15 years have failed to show evidence of cancellous bone loss in PHPT. In some reports, cancellous bone is not only preserved, but may even be increased.[21,22] Several mechanisms have been proposed to explain the conservation of cancellous bone volume.[22,23] These include a coupled and balanced increase in bone turnover, increased modeling activation frequency with decreased osteoclastic erosion depth, and increased osteoblastic activity with a prolonged formation period. This results in a slightly positive balance at each bone remodeling unit favoring bone formation.

An anabolic effect of PTH on bone has been suggested by the occasional observation of osteosclerosis in the metaphyses, skull, vertebrae, pelvis and femora of adults[25,26] and in the metaphyses of children and adolescents affected with PHPT.[27] Diffuse osteosclerosis in secondary hyperparathyroidism associated with renal osteodystrophy is much more common.

Technical developments that have occurred in the field of histomorphometry in recent years allow the study of not only trabecular bone volume and turnover, but of bone microarchitecture.[28,29] Mellish et al. developed a simple method for assessing two dimensional cancellous bone structure in iliac crest biopsies.[28] The trabecular structure was subdivided into nodes (node count) and free ends (free end or terminus count), which were expressed per square mm of cancellous space. A node was defined as a point at which three or more trabeculae joined (Fig. 5). A free end was defined as the end of a trabecula that was unconnected in the plane of the section to any other trabecular element. The following struts were defined by drawing a line between the nodes and free ends: node to node, node to free end, free end to free end and cortex-derived struts. Strut lengths were measured and expressed as a percentage of the total strut length and per square mm of cancellous space. A greater node count and node-to-node strut length indicated a higher degree of connectedness. The greater the connectedness, the greater the ultimate compressive strength tolerated at that site. This type of analysis provides important quantitative information concerning the changes in trabecular structure with age, in disease and in response to various drugs.

Fig. 5

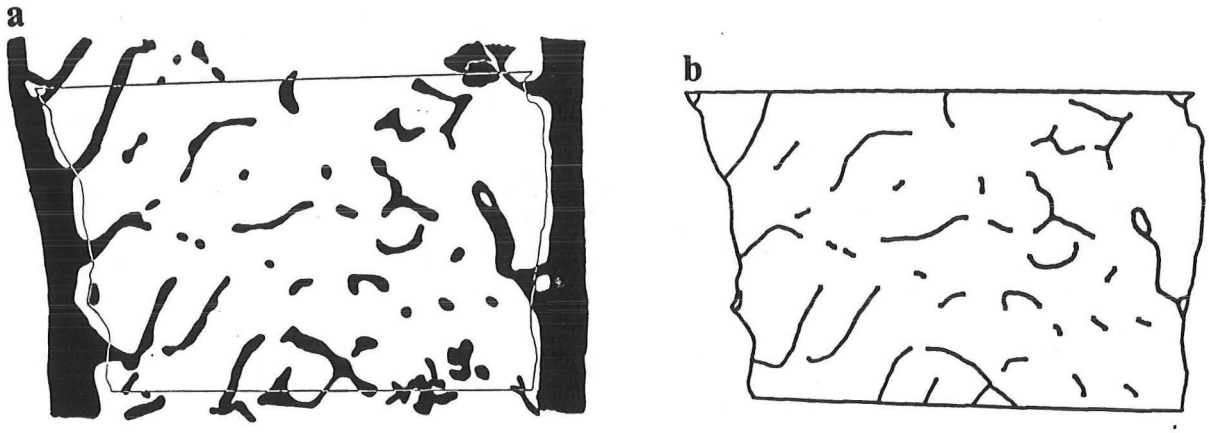
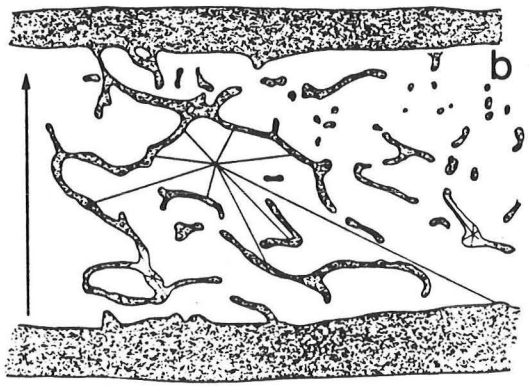


FIG. 5 (a) Diagrammatic representation of the television image of the section to be measured. The black line shows the boundary surrounding the area to be measured. Note the upper and lower boundaries have been moved from the edge of the section to avoid artifacts. (b) The final image after measurements have been made.

Another new stereologic parameter which can describe structural changes of trabecular bone is the star volume. It is defined as the mean volume of all the parts of an object which can be seen unobscured in all directions from a particular point inside the object (Fig. 6). The star volume provides an estimate of the marrow space devoid of trabeculae. It increases with age, in women more so than in men, because of a loss of structural bone components.

Fig. 6



Using trabecular strut analysis, Parisien et al. examined iliac crest biopsies from 37 patients with PHPT and compared them to those from 24 age-matched cadaveric subjects.[30] This group had previously demonstrated that patients with mild PHPT had increased cancellous bone volume due to a greater number of trabeculae.[22] Now, they were able to show that total strut length, number of nodes and node-to-node strut length were significantly higher in patients with PHPT than in controls, indicating greater connectivity (Fig. 7).[30]

Fig. 7

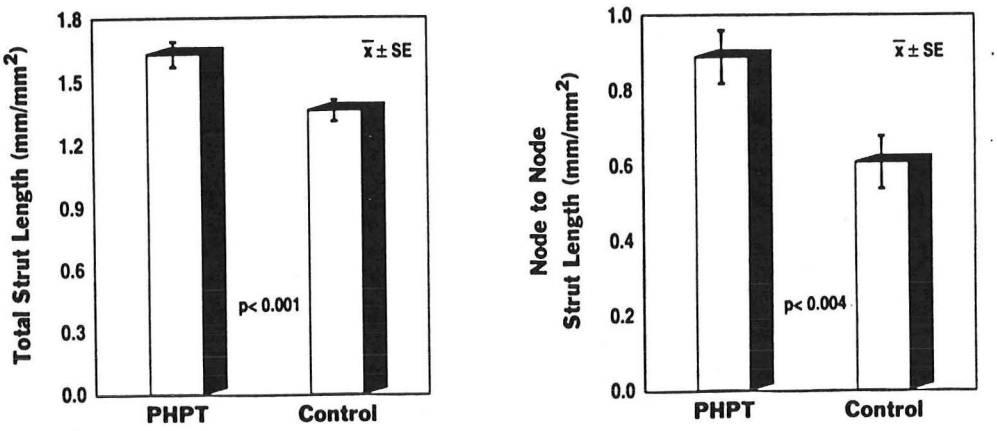


FIG. 7 Indices of trabecular structure in patients with mild primary hyperparathyroidism and controls. The higher total strut length and node to node strut length in the hyperparathyroid patients indicate higher camcellous bone volume and trabecular connectivity, respectively.

Earlier, these authors had shown that the normal age-related decrease in trabecular number and increase in trabecular separation did not occur in PHPT.[22] Now they demonstrated that the number of nodes and the node/terminus ratio did not decrease with age in PHPT as it did in normal subjects, thus indicating that age-related bone loss in PHPT occurs without significant loss of trabecular continuity.[30] Two-dimensional strut analysis had provided the first quantitative documentation of the high degree of trabecular connectivity that is preserved in PHPT.

The proposed explanation for the maintenance of trabecular connectivity is that in PHPT, age-related bone loss is characterized mainly by thinning of trabecular plates, not by perforation. This pattern resembles that observed in normal aging men and is likely to be associated with preserved bone mass and also structural integrity. This mechanism contrasts with bone loss in aging women, which is characterized by trabecular perforation and plate loss. The decreased resorption depth observed by Ericksen et al. could provide a possible mechanism of protection of trabecular plates against perforation.[23]

Paradoxically, it has been shown on the endocortical surface, in contradistinction to the trabecular envelope, resorption depth is increased in PHPT.[31] This coupled with enhanced subperiosteal erosion appears to be the mechanism of cortical thinning known to occur in this disease.

To summarize the work of Parisien et al., it appeared that the effect of increased endogenous PTH was to maintain cancellous bone volume by a mechanism that allows the relative preservation of trabecular architecture. Thus, trabecular plates, although becoming thinner with age, do not perforate and remain connected.[21,22,30] That the microarchitecture of cancellous bone is maintained was further demonstrated by comparing normal and hyperparathyroid postmenopausal women matched by age and by number of years postmenopausal. A higher cancellous bone volume and an absence

of age-related loss of bone structure and trabecular connectivity was again demonstrated in the hyperparathyroid group.[32,33] These findings were supported by similar observations from Meunier's laboratory[34,35] and strongly suggest that mild PHPT may protect women against the postmenopausal deterioration of cancellous architecture and, possibly therefore, against vertebral fractures.

Further support for the findings of the Columbia group (Parisien et al.) has been provided by a recent Danish study of cancellous bone structure and turnover in 69 patients with mild PHPT, who were compared to 30 age- and sex-matched normal subjects.[36] Normal postmenopausal women (age $\geq$50 yr) had lower trabecular bone volume and higher inter-trabecular distance (star volume) than normal premenopausal women (age <50 yr). This difference was not found in women with PHPT indicating that women with PHPT in some way seem to be protected from the accelerated bone loss seen in normal women in connection with the menopause. Due to an enhanced activation frequency, trabecular bone remodeling was increased in PHPT. This remodeling was balanced, and except for slight thinning of the trabeculae, no changes in trabecular bone structure were found. There was no indication of accelerated bone loss. On the contrary, the data suggested conservation of trabecular bone volume with aging in PHPT. TBV was not different from normal controls. The marrow spare star volume was not increased in PHPT and did not increase across the menopause as it did in normals.

Thus, the cumulative data assembled from several laboratories on bone mass and structure in PHPT have shown that there is maintenance or even an increase of cancellous bone volume with a conservation of cancellous microarchitecture and preserved or even increased connectivity of trabecular plates.[37] These findings are observed not only in men and premenopausal women, but also in postmenopausal women with PHPT, at a stage when estrogen deficiency would normally predispose to accelerated bone loss. These data suggest that moderately increased endogenous PTH levels may protect against cancellous bone loss.

The preservation of the trabecular lattice in PHPT has been associated with an increased compressive strength in iliac crest biopsy cores compared to normal controls.[38] Moreover, Parfitt's group has shown that the prevalence of vertebral fractures in postmenopausal women with mild PHPT was no greater than, and probably less than the rates of fracture observed in a historical control group of age-matched women (Table 2).[29]

Table 2. Prevalence of Vertebral Fractures in white women (Age: 55-74 years)

Asymptomatic PHPT (N=72)	Healthy Controls (N=63)
2.8%	5.2%

The increased rates of vertebral fractures reported in previous series[20,40,41] were attributed to inappropriate control groups, the influence of referral selection bias (e.g. patients

referred to Mayo Clinic with coexistent osteoporosis), the inclusion of patients with severe disease, and the effect of geographic differences in vitamin D nutrition on the expression of disease.

The data that moderately increased endogenous PTH levels may protect against cancellous bone loss is fairly convincing. There is nevertheless a concern that reductions in cortical bone mass consistently observed in this disease[21,42-44] may offset the potential utility of PTH as a therapeutic agent in postmenopausal osteoporosis. Bone mineral content of the radius in PHPT is often less than 80% of normal values. Following parathyroidectomy, there is usually an increase in radial bone density of 5-7% seen in the first year, but no further improvement thereafter.[42,43] These points are illustrated in Figs 8 and 9 from ref 42.

Fig. 8

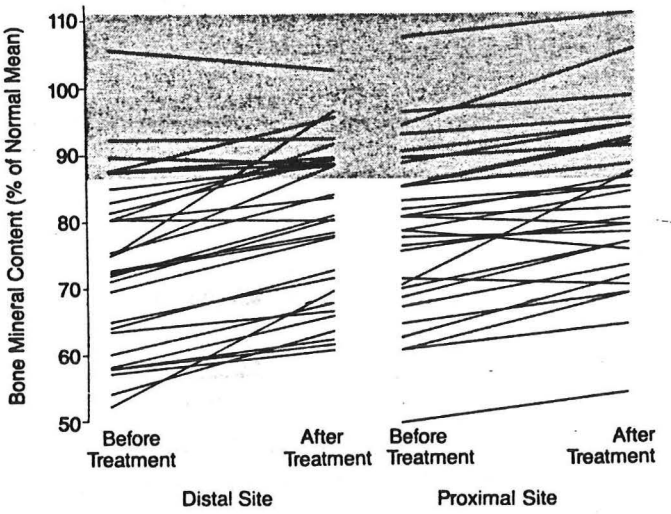


Fig 8 —Bone mineral content before treatment and one year after surgery. Though bone mineral content increased in most patients, it was usually not within normal range at one year.

Fig. 9

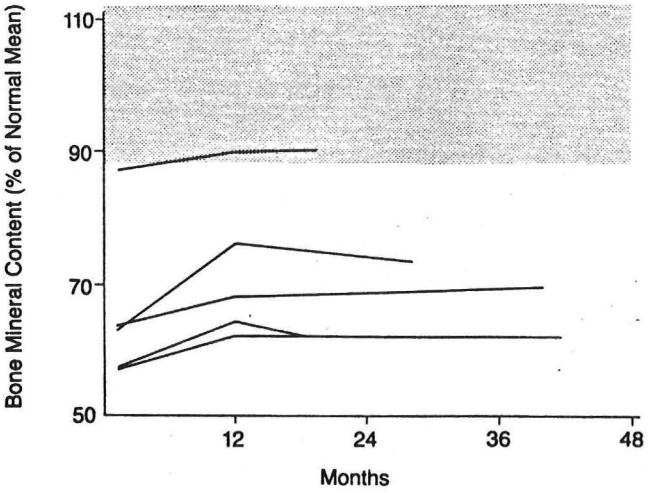


Fig 9 —Long-term changes in bone mineral content in five patients. After initial increase, bone mineral content tended to remain lower than normal for up to 40 months.

Any potential treatment of osteoporosis with PTH would have to in some way protect against cortical bone loss.

IV. In Vitro Studies of PTH Action

Despite the fact that the major action of PTH in bone is to stimulate osteoclastic bone resorption, a compelling body of evidence has been published over the last two decades indicating that cells of the osteoblast lineage are the principal, but not necessarily the only target.[37] The concept that osteogenic cells, rather than the osteoclast, were the primary targets for PTH as well as for other agents that stimulate bone resorption was first formally proposed in a classic paper by Rodan and Martin in 1981.[45] This concept of "reverse coupling" received experimental support when investigators developed successful techniques for studying functionally viable osteoclasts

in vitro. Thus, Chambers and his colleagues[46] showed that when osteoclasts were physically separated from other bone-derived cells on a devitalized slice of bone, they did not respond to PTH. However, when primary cultures of osteoblasts or osteoblast-like cells were added to the culture, PTH then induced an increase in resorption. Chambers' group and others suggested the existence of a soluble osteoclast-stimulating factor(s) released by osteoblasts in response to PTH.

In the past couple of years, there have been a few reports of PTH receptors also in osteoclasts.[37] PTH may have an inhibitory effect on bone resorption by purified cultures of osteoclasts. It thus appears that both osteoblasts and osteoclasts express functional PTH receptors. This raises the possibility of dual regulatory mechanisms for PTH action on bone with the peptide perhaps exerting a direct inhibitory effect on the osteoclast, and an indirect stimulatory effect mediated by osteoblasts.

Anabolic Effects on Osteoblasts

Using cultured embryonic mouse radii, Herman-Erlee et al. showed that synthetic bovine PTH 1-34 ($\leq 5 \times 10^{-9}$ M) increased osteoblast number and osteoid formation in more than half of the samples studied. However, a number of investigators have shown that PTH decreases collagen synthesis, alkaline phosphatase activity and osteoclacin synthesis by osteoblasts in various models.[37] Although PTH decreases osteoblastic synthesis and secretion of collagen and other peptides, it appears to stimulate osteoblast replication as measured by the incorporation of ^{3}H-thymidine into DNA in bone organ cultures.[48] This observation suggests that the anabolic effect of PTH might be mediated by an increase in the proliferation of osteoblast precursors or osteoprogenitor cells.[49] In general, the effects of PTH on mitogenesis are associated with a reduction in the expression of the osteoblast phenotype;

However, an anabolic effect of PTH on bone that is independent of its mitogenic action should not be discounted. Canalis and his colleagues have demonstrated that, as is the case in vivo (see below), the manner in which PTH is administered in vitro profoundly influences its effects on bone formation.[48] Thus, in cultured fetal rat calvariae, continuous treatment with PTH for 24-72 hr inhibited type I collagen production, but transient exposure for 24-hr followed by incubation in PTH-free medium resulted in a stimulation of collagen synthesis.[48] The collagen synthesis stimulated by PTH was not blocked by hydroxyurea, indicating that it was not dependent on the mitogenic effect of PTH. These results imply that PTH can have stimulatory effects on differentiated osteoblasts as well as on the differentiation of preosteoblasts.

Recent studies in several laboratories have provided convincing evidence that the PTH-induced stimulation of bone formation is mediated by local growth factors, with IGF-1 and TGFβ being the primary candidates. IGF-1 enhances collagen production by stimulation of osteoblast replication and increasing the synthesis of type I procollagen mRNA by differentiated osteoblasts.[50] PTH stimulates the release of IGF-1 from fetal rat calvariae[48] and also increases the IGF-1 in osteoblast-enriched primary cell cultures.[51] Moreover, the stimulatory effect of transient PTH administration on type I collagen synthesis in fetal rat calvariae is abolished by IGF-1 neutralizing antibodies, an effect that

is independent of PTH's mitogenic action, which was unaffected by the antibodies.[48] The ability of PTH to stimulate IGF-1 in fetal rat bone exceeded that of GH.[51]

Another local factor produced by bone cells that is abundant in bone matrix is TGF-β.[52] TGF-β regulates replication, collagen synthesis, and alkaline phosphatase in osteoblast-enriched cell cultures. TGF-β content increases in bone culture medium after PTH treatment (more from matrix resorption than direct osteoblast synthesis), and locally released TGF-β could account for the anabolic effects of PTH on bone. PTH is known to increase TGF-β binding to its receptors in osteoblast enriched cultures.[52]

Anticatabolic Effects on Osteoclasts

While studies on the anabolic action of PTH have necessarily focused on osteoblasts and bone formation, we should not overlook the potential that "anticatabolic" effects might also alter the balance between formation and resorption in favor of the former. Duong et al. reported that bovine PTH 1-34 had an inhibitory effect on bone resorption by purified cultures (osteoblast-deficient) of chick osteoclasts.[53] (A stimulatory response was observed in less pure populations with a greater density of contaminant osteoblasts). Using the resorption pit assay, Dempster's group has found evidence for an inhibitory effect on osteoclast function even in the presence of osteoblasts.[54] While osteoclasts were activated by PTH and the average number of pits per resorption focus was increased, the area of the individual resorption pits was substantially reduced. Such an effect is consistent with histomorphometric data in patients with PHPT in which there is increased activation frequency of bone remodeling units but a reduction in the depth of the resorption lacunae.

In conclusion of this section, these basic studies have elucidated several possible mechanisms for a direct anabolic action of PTH on the skeleton. These include cytokine-mediated stimulation of osteoblastic proliferation and activity and possibly, inhibition of osteoclast activity. The remainder of the review will focus on PTH action in intact animals and humans.

V. Animal Data

Physicians have traditionally considered PTH to be an agent that is catabolic to the skeleton. However, as early as 60 yr ago, Albright and his colleagues[55] and later Selye[56] noted that the peptide extract could also be anabolic to the skeleton, producing increases in bone tissue in animals. The majority of data supporting an anabolic effect of PTH comes from animal studies. Although the rat has been the principal species in which the effects of PTH have been evaluated using a variety of models of bone loss, data are also available for the dog. The early results on the anabolic effect of PTH on animal skeletons have been extensively reviewed previously by Parsons.[57]

During the last decade, improved analytical techniques and the wide availability of purified PTH fragments led to a renewed interest in the study of the anabolic action of PTH on bone based on sensitive quantitative determinations of the skeleton and its modeling processes. The apparent success of daily administration of low doses of PTH

to humans,[58] and in controlled data using dogs,[59] further prompted this interest. Tam et al.[60] reported an increase in bone formation surface and trabecular bone volume, but not in resorption surface in rats treated intermittently with 0.4 to 102.4 U/rat/day of PTH for 12 days (Fig 10).

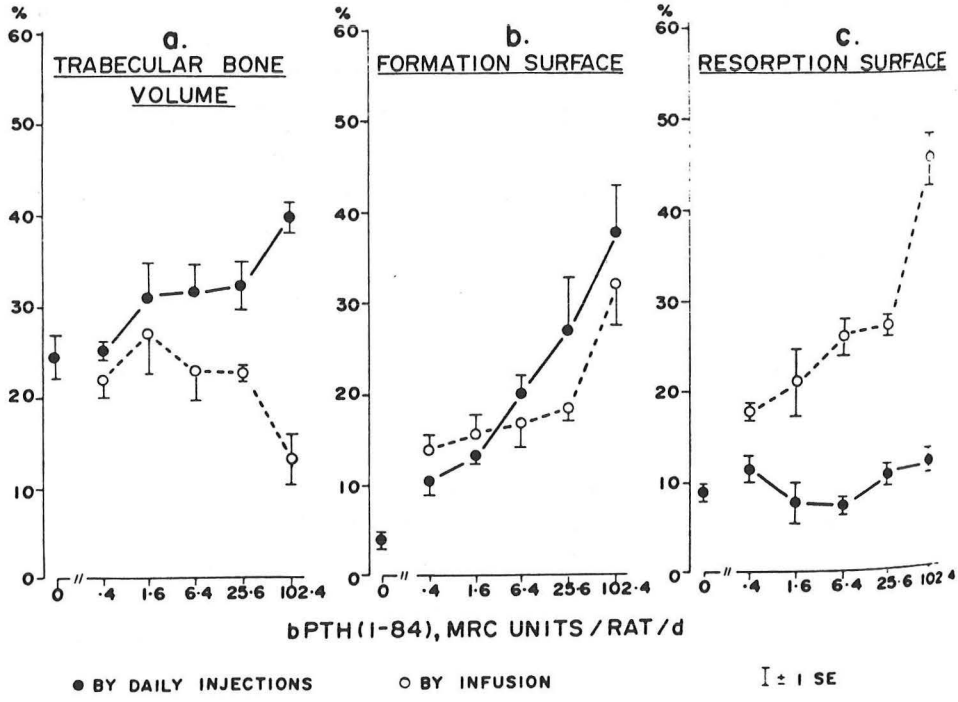


FIG. 10 Morphometry of the lower metaphysis of the left femur in rats receiving bPTH-(1-84) by intermittent sc injection or continuous infusion.

The trabecular bone volume, expressed as the percentage of the total bone tissue volume (matrix plus marrow), showed an increase when the hormone was injected and a decrease when the hormone was infused (a). The formation surface, expressed as the percentage of the total bone surface, showed a PTH dose-dependent increase, which was the same for both the injected and the infused groups (b). The resorption surface, expressed as the percentage of the total bone surface, showed no response to PTH up to 102.4 MRC units/rat·day, but a dose-dependent increase was observed when the hormone was infused (c). There were four rats in each of the infused groups and five in each injected group.

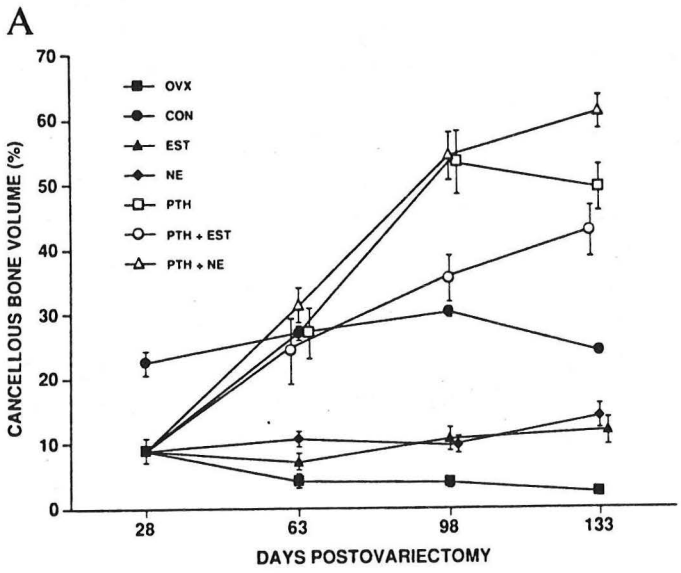
Tam's results showed that PTH could stimulate formation, without necessarily stimulating resorption. In contrast to the intermittent injections, a continuous infusion of a similar dosage of PTH was associated with a decrease in trabecular bone volume, owing to resorption exceeding formation (Fig 10).[60]

Other observations from numerous rat studies reviewed in reference 37 include the following. There was a significant dose-dependent increase in cancellous bone, but not in cortical bone, after PTH injection. The anabolic action of PTH when given by daily injection was consistent and proportional to the dose used in the experiment. Both male and female rats respond to PTH treatment equally well and the age of the rats that respond to PTH can vary from prenatal to 2 yr. An interruption in the daily therapy of 4 days results in significant loss of the stimulated bone formation activity, and a consequent loss of bone mass can be observed as early as 8-12 days after withdrawal of the hormone. The notion, interpreted from human studies, that cancellous bone is increased by PTH at the expense of cortical bone[58] has not been seen in the rat. The dietary calcium intake is proportionally much higher in the laboratory rat than in humans, making it unnecessary to remove calcium from cortical bone to support cancellous bone formation elsewhere. The bone formed under stimulation by PTH appears to be of normal quality. The increases in mass are accompanied by an improvement in biochemical strength of vertebral bodies, and this remains even after normalization for both cross-sectional area and bone mass.[61]

Early experiments with PTH were performed in intact animals with normal skeletal status. Despite earlier concerns regarding their suitability, several rat models of osteoporosis have been developed, indicating that rats may indeed be useful in the study of pathogenesis and treatment of osteoporosis in humans. For example, in rats, ovariectomy, orchidectomy, and immobilization of limbs can induce bone loss in the skeleton with increments in bone turnover as is seen in humans. The anti-resorptive agents used in humans, estrogen, calcitonin, and bisphosphonates, reduce bone turnover and prevent further bone loss in the rat models as they do in humans without substantially restoring bone mass or architecture. When tested in various rat models of osteoporosis, intermittent injection of PTH both prevents loss and restores bone mass caused by oophorectomy.[62,63]

Thus, Wronski et al. showed that treatment of ovariectomized rats with estrogen of risedronate alone depressed bone turnover and prevented additional cancellous bone loss from occurring during the treatment period (Fig 11). However, these therapeutic agents failed to restore lost bone in OVX rats to control levels. In contrast, OVX rats treated with PTH alone exhibited a marked stimulation of bone formation which resulted in augmentation of cancellous bone mass to a level 2-fold greater than the vehicle-treated control rats. Concurrent treatment of OVX rats with PTH + estrogen as well as PTH + risedronate also effectively reversed cancellous osteopenia, but did not appear to be more beneficial to the estrogen-depleted skeleton than treatment with PTH alone. The results indicated that PTH was a powerful stimulator of bone formation and completely restored lost cancellous bone in osteopenic OVX rats. Furthermore, the bone anabolic effects of PTH were much more pronounced that those of estrogen or bisphosphonates. These findings in an animal model of estrogen depletion provided support for PTH as a potentially effective treatment for oophorectomized and postmenopausal women with established osteoporosis.

Fig. 11



The mechanism for the enhanced cancellous bone volume achieved by PTH is shown in Fig 12.[62] Unlike estrogen and risedronate which depress the bone formation rate, PTH stimulates the bone formation rate further.

Fig. 12

RESTORATION OF LOST BONE MASS BY PTH

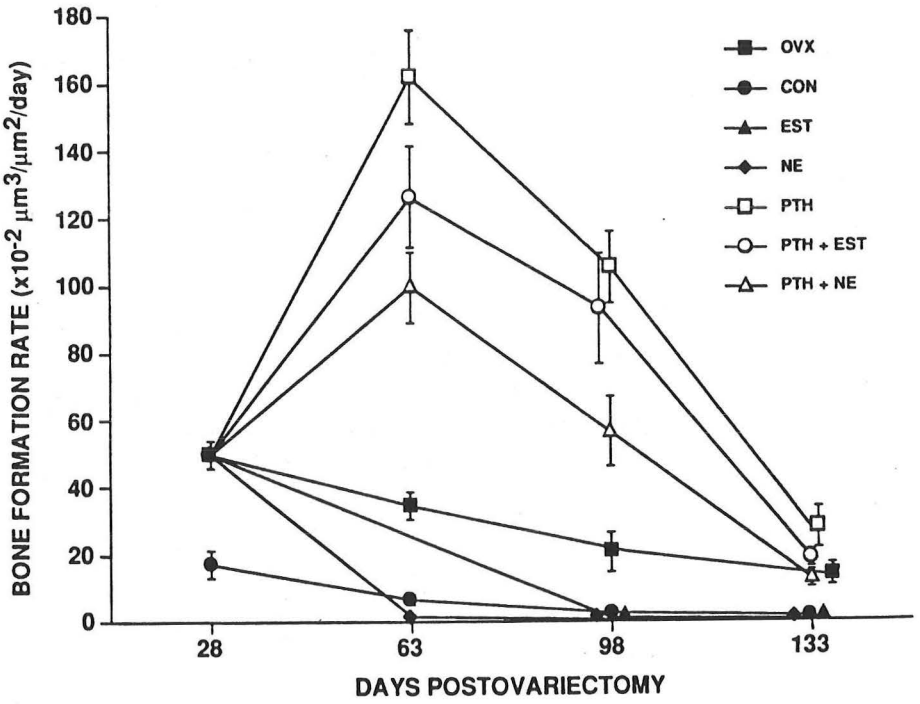


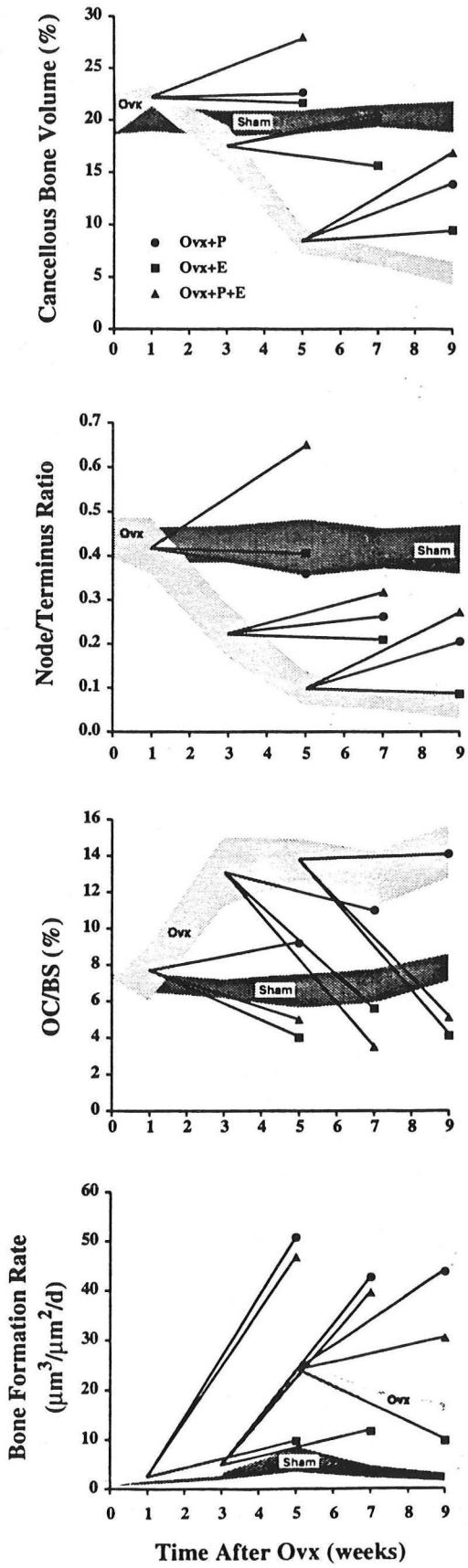
FIG 12 Bone formation rate (tissue level, surface referent) in the proximal tibial metaphysis as a function of time postovariectomy. See legend to Fig. 1 for details.

Conventional treatment with antiresorptive agents will not restore lost bone mass or structure. Addition of an anabolic agent to the antiresorptive treatment should in theory achieve both a reduction in bone resorption and an enhancement of bone formation. The above findings were confirmed and extended by Shen et al.[63] who studied the effects of treatment of bone loss in ovariectomized rats with a combination of estrogen and PTH. The results indicated that such combined treatment was not only capable of increasing cancellous bone volume, but also of re-establishing trabecular connectivity (Fig 13).[63] This ability to restore bone mass and structure previously lost has significant implications for the treatment of established osteoporosis in humans, especially for the disease among postmenopausal women, the most common form of the disorder, estimated to affect some 20 million women in the United States. In this study, estrogen treatment conferred an additional advantage over PTH alone, with respect to gain in cancellous bone volume and connectivity. Moreover, in human studies, it might protect against cortical bone loss.

The discrepancy of the findings between intermittent PTH injections and severe PHPT where bone loss was evident, remained a puzzle for a long time until experiments conducted in dogs and rats suggested convincingly that the difference may, at least in part, relate to differences in the modes of administration of PTH. Podbesek et al. compared daily SQ injections of hPTH 1-34 (15 U/kg/day) with continuous infusion (4.5 U/kg/day) in greyhounds.[59] Daily injection of PTH significantly increased indices of bone formation (osteoid surface, plasma alkaline phosphatase, and rate of skeletal accretion of calcium) and resorption (number of osteoclasts and resorption surfaces) and increased trabecular bone volume in the ilium. On the other hand, continuous infusion of PTH, using similar doses of PTH, did not significantly increase trabecular bone volume or calcium accretion rate, and only osteoclast surfaces were clearly increased. Tam et al. gave rats various doses of bovine PTH 1-84 by either daily injection or continuous infusion.[60] In this experiment, bone formation surfaces were elevated by both methods of administration, but resorption increased only if PTH was given by continuous infusion. The net result was an increase in cancellous bone volume when PTH was given by daily injection. Infusion, on the other hand, maintained cancellous bone volume at low doses but caused a decrease in cancellous bone volume at high doses. Thus, the destructive effects of PHPT on bone, as reported in early studies of the disease, would be comparable to high dose-constant infusion experiments, while the more recent findings that trabecular bone mass is preserved in mild PHPT may be the result of the lower endogenous dose of PTH.

Concerning the mechanism of the anabolic action of PTH, prior resorption does not appear necessary since stimulation of bone formation without resorptive activity can be seen in rats when PTH is administered by daily

Fig. 13



injection.[60] Furthermore, co-administration of a resorption inhibitor, calcitonin, bisphosphonate or estrogen, with PTH fails to block the anabolic response of bone to PTH.[62,63] In fact, as noted above, the combined effects of estrogen administered with PTH are significantly better than either alone, both in terms of bone mass and trabecular structure.[63]

As discussed earlier, according to histomorphometric observations, PTH increases bone formation by extending mineralization surfaces and/or the rate of mineral apposition. This is likely the result of increased osteoblastic precursor proliferation and/or maturation. There is some in vitro evidence that IGF-1 might be a mediator for this effect. It has been reported that the anabolic effect of PTH depends on the presence of GH, a significant positive modulator of IGF-1 secretion.[64] As discussed, GH and IGF-1 would be potential candidates for treatment of osteoporosis, but could be associated with undesirable side effects. In a sense, PTH may serve as a bone-seeking, growth factor stimulator capable of increasing the local concentration of IGF-1 in bone.

VI. Human Studies

Despite the animal data showing as early as 1929, that PTE and PTH administration could have an anabolic effect on the skeleton, reports of preliminary human trials investigating PTH administration in osteoporotic patients did not appear until the mid 1970's.[65,66] This was at least in part because of unavailability of the material until hPTH 1-34 was synthesized at the MGH in 1974.[67,68] Bovine PTH had been shown to elicit neutralizing antibodies after short-term administration.

In preliminary data obtained from balance and kinetic studies examining the effects of short-term sc hPTH 1-34 administration, high doses (750 U daily) stimulated resorption in excess of formation, whereas lower doses (450 U) caused an overall improvement in calcium balance, with increases in intestinal Ca absorption and bone accretion.[66,69] These early data were comparable to those obtained in both the rat and dog experiments discussed earlier.

Combined results of a small multi-center trial were published by Reeve et al.[58] A total of 21 osteoporotic patients (16 women and 5 men) with either vertebral or limb fractures were enrolled in 7 centers. The patients and the therapies were somewhat heterogeneous (1 taking vitamin D, 3 taking estrogen and 1 man receiving testosterone). Data were analyzed with each patient serving as his or her own control. Patients had baseline investigations including calcium balance, a radiocalcium kinetic study to allow determination of new bone formation and calculation of bone resorption rate, cortical densitometry of radius or femur by single photon absorptiometry, as well as a transiliac crest bone biopsy. Studies were repeated after the treatment period.

Patients were treated with once daily sc injections of synthetic hPTH 1-34 (500 U) for periods of 6-24 months. No serious side effects of the treatment were noted, with only transient hypercalcemia in four cases. Overall, not surprisingly, considering the heterogeneity of the group, there was a large inter-individual variation in responsiveness

to the treatment protocol. Mean serum alkaline phosphatase increased 15% above baseline in association with increases in urinary hydroxyproline, calcium and phosphate excretion. Striking increases in new bone accretion measured using a ^{47}Ca-kinetic method were noted (mean increment 144%), with lesser increments in resorption rate. In 9 paired iliac biopsies on which quantitative analysis could be performed, increases in cancellous bone volume were seen (mean increase 92%). There were also increases in osteoid covered cancellous surfaces (mean increase 50%). A close correlation was found between ^{47}Ca accretion rate and cancellous bone volume at the end of the treatment period.

In contrast, net intestinal calcium absorption did not increase consistently and, more importantly, overall calcium balance did not change positively. The former finding might have been due to a lack of increase in 1,25-$(OH)_2$D. In two normal subjects, in whom the kinetics of PTH 1-34 were studied after injection, rapid clearance from the circulation was observed with return to baseline levels after 6 h (RIA). The lack of positive calcium balance in association with prominent increments in trabecular bone volume, led to the thesis that exogenous PTH administration in this regimen increased cancellous bone mass at the expense of cortical bone loss, a result shown in a subgroup of patients in whom bone mass had been measured in the femur.[70] In order to guard against this potential problem, investigators sought to use human PTH in combination with 1,25-$(OH)_2$D or with an antiresorptive agent.

Combination with 1,25-$(OH)_2$D

To address concern regarding lack of consistent improvement in calcium absorption, Slovik and co-workers[71,71] added daily oral 1,25-$(OH)_2$D (.25 μg - 0.5 μg) to a regimen of daily sc hPTH 1-34 (400 - 500 U/d) in 8 men with idiopathic osteoporosis (mean age 50) and 6 women with postmenopausal osteoporosis for 8-24 mos. A similar group of osteoporotic women treated with calcium of 1,25-$(OH)_2$D alone served as controls. In 4 of 4 male subjects in whom vertebral density measurements were available, trabecular BD (by single energy computed x-ray tomography) increased on average 98% after 12 mos of therapy. (Fig.14). Increments in vertebral bone density also occurred in women, but were not as high as those observed in men.[72] Radial bone density by SPA did not change. In contrast, in those women treated with calcium or 1,25-$(OH)_2$D alone, cortical bone density in the radius decreased. Calcium and phosphorus balance improved in 4 of 4 male patients associated with increments in dietary calcium and phosphorus absorption. The conclusion from these studies was that the combination of hPTH 1-34 and 1,25-$(OH)_2$D had greater anabolic effects on bone than either

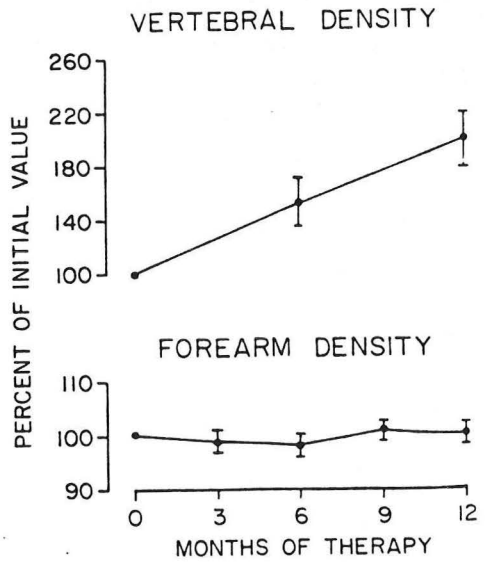


FIG. 14 Changes in trabecular bone density of lumbar vertebrae (*top*) and of cortical bone density of the radius (*bottom*) in osteoporotic men treated with daily sc injection of 400–500 U hTPH(1–34) plus daily ingestion of 15–30 mmol calcium and 0.25–5.0 μg 1,25$(OH)_2D_3$. [Reproduced with permission from D. M. Slovik *et al.*: *J Bone Miner Res* 1:377–381, 1986 (225).]

agent alone in osteoporotic men and women. Given that cortical bone density did not decrease and that calcium balance increased, the authors concluded that this regimen resulted in a true increase in total bone mass without sacrificing cortical bone.

More recently, Neer et al. published a controlled trial of 30 women with postmenopausal osteoporosis, half of whom received 400 - 500 U hPTH 1-34 plus 0.25 mcg calcitriol/day, while the control group received only calcium supplements.[73] Total spinal bone mineral density of PTH-treated women by DPA increased 12% over 1-2 yr with no change in the control group. In an unspecified cortical site, BMD decreased by 5.7% in the PTH-treated group vs only 1.7% in the control group. Furthermore, these investigators noted that after 6-12 months, spinal bone mass stopped increasing for reasons that are unclear. There are currently no long-term data on the effects of PTH treatment > 24 months. There was no incidence of hypercalcemia, hypophosphatemia, or declining creatinine clearance.

Combination with Estrogen

Reeve and colleagues studied 12 postmenopausal osteoporotic women with vertebral fractures in an open, non-randomized 1-yr protocol.[74,75] The subjects received hPTH 1-34 and estrogen for 8 of 12 months (in 9 patients) or nandrolone (25 mg every 3 weeks) in 3 patients. One of the patients stopped her estrogen therapy. By QCT, vertebral bone density increased 56% above baseline, with no change in radial bone density. Radioisotopic measures of bone formation showed marked increments. Calcium balance improved in most patients, with the mean increment (+68 mg/d) approaching significance. On histomorphometry of iliac crest biopsy, cancellous bone area increased because of a greater number and thickness of trabeculae, and there was a 20% reduction in trabecular separation (Fig. 15).[75]

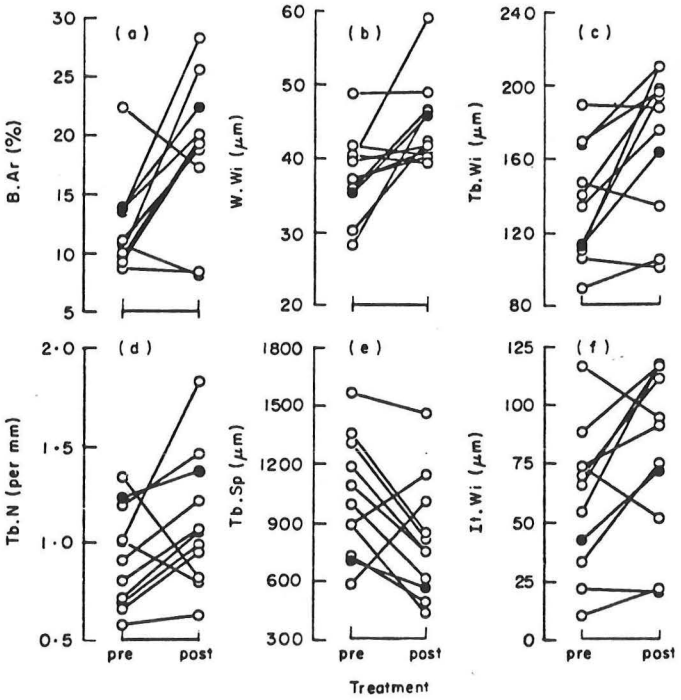


FIG. 15 Changes in trabecular bone structure in iliac crest biopsies of 11 osteoporotic women treated for 12–14 months with a combination of 460–740 "house" units of hPTH(1–34) and hormone replacement therapy (conjugated equine estrogens + norgestrel) for 12–14 months. The patient represented by *black circles* discontinued hormone replacement therapy. B.Ar, Cancellous bone area; W.Wi, wall width of trabecular bone packets; Tb.Wi, trabecular width; Tb.N, trabecular number; Tb.Sp, trabecular separation; It.Wi, interstitial width. The increments in B.Ar, W.Wi, and Tb.Wi were statistically significant ($P < 0.05$–0.01) for the group. [Reproduced with permission from J. N. Bradbeer *et al.*: *Clin Endocrinol (Oxf)* 37:282–289, 1992 (232). ©Blackwell Scientific Publications.]

More recently, Lindsay's group has initiated a controlled, clinical trial of the effects of PTH 1-34 in estrogen-treated osteoporotic women.[76,77] The idea was that estrogen would block resorption effects of PTH, particularly cortical bone loss. Patients with

postmenopausal osteoporosis were treated with estrogen until bone density stabilized (minimum of 1 year). These patients were then randomly allocated to treatment with daily hPTH 1-34 400 U (in addition to estrogen) or control (estrogen alone). Groups were well matched for demographic and biochemical variables as well as bone density. Biochemical indicators of bone formation (osteocalcin, alkaline phosphatase) and bone resorption (hydroxyproline, pyridinoline and tartrate resistant and phosphatase) increased during PTH 1-34 therapy. In the PTH-treated group, bone density (Hologic QDR-1000) increased progressively in the lumbar spine by 4.6% at 6 months to 10.1% after 18 months of treatment, whereas no change was seen in the estrogen-treated control group ($P < .004$). There was also a small increment in bone mass of the proximal forearm in the PTH treatment group (3.2%) compared with a small loss (-1.2%) in the control group ($P < .002$). Minimal femoral neck decreases (-1.5% in treatment and -2.6% in controls) occurred in both groups. Midway through this 3 year study, it appears that PTH 1-34 increases vertebral bone mass without detrimental effects on cortical sites. Moreover, there was no evidence of a plateau effect as observed after 1 year in Neer's study of PTH effect on women.[72]

VIII. Conclusion

Possibly, because of difficulty obtaining sufficient material (PTH 1-34), or lack of support for this type of clinical research, very few good clinical studies of the effect of PTH on bone have been done to date. Most have non-homogeneous study groups, no control subjects, no assessment of fracture incidence, and none have been carried on for more than 24 months. Neer, and particularly Lindsay, have made some progress in this regard. Although the studies performed have been very preliminary, they do appear to offer some hope that PTH alone or in combination with other agents may yield a meaningful anabolic effect on the skeleton. It will be of interest to see if PTH can help reverse the loss of trabecular connectivity seen in postmenopausal osteoporosis in addition to increasing trabecular volume. And of course, the bottom line will be, if changes in bone mass and structure are positive, will this translate into a reduction in fracture frequency?

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