■ INSTRUCTIONAL REVIEW - RESEARCH

Parathyroid hormone 1-34 and skeletal anabolic action

THE USE OF PARATHYROID HORMONE IN BONE FORMATION

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Intermittently administered parathyroid hormone (PTH 1-34) has been shown to promote bone formation in both human and animal studies. The hormone and its analogues stimulate both bone formation and resorption, and as such at low doses are now in clinical use for the treatment of severe osteoporosis. By varying the duration of exposure, parathyroid hormone can modulate genes leading to increased bone formation within a so-called 'anabolic window'. The osteogenic mechanisms involved are multiple, affecting the stimulation of osteoprogenitor cells, osteoblasts, osteocytes and the stem cell niche, and ultimately leading to increased osteoblast activation, reduced osteoblast apoptosis, upregulation of Wnt/β-catenin signalling, increased stem cell mobilisation, and mediation of the RANKL/OPG pathway. Ongoing investigation into their effect on bone formation through 'coupled' and 'uncoupled' mechanisms further underlines the impact of intermittent PTH on both cortical and cancellous bone. Given the principally catabolic actions of continuous PTH, this article reviews the skeletal actions of intermittent PTH 1-34 and the mechanisms underlying its effect.

Introduction

Human parathyroid hormone (PTH) is an 84-amino acid polypeptide secreted from the parathyroid gland. Acting predominantly on the skeletal system and renal tubules, the hormone modulates serum calcium and phosphate.[1-3] Teriparatide (PTH 1-34) is the N-terminal fragment of the intact hormone and is approved for use in the treatment of osteoporosis in both the United States and Europe.[4] During normal homeostatic conditions bone resorption is equal to bone formation,[5,6] yet PTH, when administered in both a continuous and intermittent (iPTH) fashion, alters this balance leading to increased bone turnover. Moreover, when administered intermittently, there is an early stimulation in bone formation without an increase in bone resorption, termed the 'anabolic window'.[7,8] Both animal and human studies have supported these findings in young and old subjects. Studies, including those conducted on postmenopausal women, demonstrate a profound anabolic effect of PTH in areas of increased bone turnover such as at fracture sites and at the acute bone-implant interface.[9-12]

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Both PTH 1-34 and PTH 1-84 are used in the treatment of osteoporosis in postmenopausal women, with both having been shown to reduce the risk of new vertebral fractures.[13-16] In addition to these agents, parathyroid hormone-related protein (PTHrP) has gained more attention in the last decade. This factor is isolated from tumours associated with the paraneoplastic syndrome of humoral hypercalcaemia of malignancy, and shares homology with PTH 1-34.[17] Shown to be integral to bone formation, PTHrP acts via both PTH-Receptor1 (PTHR1) and an unrelated PTHrP-specific osteoblast surface receptor.[17,18] PTHrP mediates osteoblast differentiation and proliferation via a number of pathways shared with PTH (extracellular signal related kinases, cyclin dependent kinase inhibitor (ERK,P27) ERK, P27) and receptor activator of nuclear kappa B ligand/osteoprotegerin (RANKL/OPG); though unlike teriparatide, its use remains off-label. Nonetheless, the clinical applications of PTHrP may be further reaching than those of teriparatide alone, with rodent and canine studies outlining the

regulatory role of PTHrP on cartilaginous proliferation at the growth plate.[19-20]

The so-called 'anabolic window' synonymous with intermittent PTH 1-34 is a result of multiple mechanisms, the net effect being a positive balance between formation and resorption within the bone remodelling unit (BMU). Intermittent dosing has been shown to have an effect on both remodelling- and modelling-based bone formation at cortical and cancellous surfaces.[21-22] Although highlighting the differences between continuous and intermittent administration, this review focuses on the mechanisms of pulsatile PTH 1-34.

Anabolic role of PTH

Osteoblast activation and stem cell differentiation. Both continuous and intermittent administration of PTH lead to increased bone turnover at trabecular and cortical sites.[21-24] However, continuous dosing results in increased osteoclast activation and lifespan, and thus enhanced endosteal resorption. In contrast, intermittent dosing results in increased trabecular bone volume. A significant proportion of this anabolism is mediated by the intermittent PTH effect on the osteoblast. The primary receptor for PTH and PTHrP is the G-protein-coupled receptor PTH1R, known to be expressed on the surface of osteoblasts, osteocytes, stromal cells, T cells and macrophages.[25-27] At low concentrations, PTH binds preferentially to PTH1R on cells of the osteoblastic lineage, thus driving osteoblastic bone formation.[28,29] Moreover, rapid degradation of the hormone ensures that osteoclastic bone resorption is not activated via this mechanism. Stimulation of PTH1R activates a multitude of G-proteins including the $G\alpha$s-mediated cascade, which leads to the conversion of adenylyl cyclase to cyclic-adenosine monophosphate and the activation of protein kinase a (cAMP/PKA pathway).[30,31] The resultant subunits translocate into the cell nucleus, leading to transcription factor phosphorylation and the expression of messenger ribonucleic acid (mRNA) responsible for the anabolic effects.

The transient upregulation of mRNAs encoding for transcription factors, cytokines and growth factors via cAMP/PKA signalling mediates a variety of the anabolic actions of intermittent PTH. Upregulation has been demonstrated to occur within the first six to nine hours following administration.[32-33] As such, intermittent PTH may lead to repeated cycles of upregulation and thus to an overall net anabolic effect. Of these multiple transcription factors, both the Notch ligand *jagged-1* and the proto-oncogene *c-fos* play substantial roles in the proliferation of osteoblasts as a result of PTH treatment.[34-36] *In vivo* studies have shown that *c-fos* expression is greatest from osteoblasts following the administration of intermittent PTH in the first 90 minutes, after which gene production was a result only of the osteoclast population.[35] Intermittent PTH has also been shown to induce the activation of runt related

transcription factor 2 (RUNX2). This transcription factor not only drives the differentiation of stem cells down the osteoblastic lineage, but it also maintains osteoblast maturity. However, continuous administration leads to rapid degradation of RUNX2 and thus reduced bone formation.[37-38] These changes to RUNX2 levels are thought to be due to alterations of the genes' stability through changes mediated by the cell cycle regulator cyclin D1.[39-40]

The role of insulin-like growth factors (IGF) on bone anabolism can also not be understated; these proteins have been shown to not only induce osteoblast differentiation of stem cells, but also to increase the activity of mature osteoblasts.[41] Intermittent PTH dosing increases the expression of IGF-1 in rodent experiments, with knockout IGF-1 animals, showing no change in bone formation when treated with intermittent PTH,[42] while human studies in postmenopausal women demonstrated similar effects with concurrent increases in IGF-2 and bone formation following short courses of intermittent PTH.[43] Similar to RUNX2, IGF not only affects osteoblast differentiation and activity, but also plays a role in cell survival via an anti-apoptotic effect.[44]

Osteoblast apoptosis. A large body of work has demonstrated that increased osteoblast number is driven by an anti-apoptotic effect and thus, increased cell survival rates.[36] Osteoporotic murine models are known to have increased osteoblast apoptosis in cancellous regions, yet this phenomenon is reversed following *in vivo* administration of intermittent TH, leading to an overall increase in bone formation.[36,45] Of note, the anti-apoptotic effect of PTH is mediated via actions on the PTHR1 at the earlier stages of osteoblast differentiation, with very little effect seen with mature cells, resulting in a net 'clearance' of older osteoblasts in preference for younger cells.[45]

In vitro work has demonstrated that the cAMP/PKA pathway is the underlying mode for this anti-apoptotic effect, leading to phosphorylation of the cellular transcription factor cAMP response element binding protein (CREB), the transcription of anti-apoptotic genes Bcl-2 and P21, and the inactivation of pro-apoptotic genes such as Bad and the apoptosis inducer Cell Cycle Apoptosis Regulator Protein (CARP-1).[36,45-48] Conversely, continuous administration results in an inhibition of RUNX2 through proteasomal degradation by Smad, thus reducing osteoblast survival.[45]

Wnt signalling. One of the major effects of intermittent PTH is via activation of the canonical Wnt pathway and, in turn, Wnt/β-catenin signalling in osteoblasts. Essential for normal bone formation and cartilage repair, the pathway not only mediates the differentiation of stem cells to osteoblasts but it also regulates the maturation, proliferation and anti-apoptosis of osteoblast precursors.[44,49-50] Canonical Wnt is imperative in PTH anabolism, whereby Wnt ligands activate frizzled receptors (Fzd) and low-density receptor proteins 5 and 6 (LRP5, LRP6). This

dimeric receptor complex reduces the proteolysis of β-catenin, resulting in its increased stability and accumulation within the nucleus. Intermittent PTH is a pathway agonist, thus, the accumulated β-catenin binds to T-cell-specific transcription factor (TCF) and lymphoid enhancing factor (LEF), displacing the Groucho repressor gene, and thereby allowing the transcription of Wnt-specific osteoblast differentiation genes.[51]

Sclerostin is a glycoprotein primarily secreted from osteocytes, and acts to antagonise the canonical Wnt pathway.[52-53] Mutations in the SOST gene and thus reductions in sclerostin levels lead to Van Buchem disease and sclerosteosis, phenotypically characterised by bone overgrowth and skeletal sclerosis. Sclerostin can occupy the Wnt ligand binding sites on LRP5 and LRP6, and therefore reduce Wnt signalling, leading to reduced bone formation. Intermittent PTH reduces SOST mRNA levels and increases bone mineral density *in vivo* via the cAMP/PKA signalling pathway downstream to PTHR1.[54,55] Moreover, transgenic mice overexpressing PTHR1 have shown reduced SOST levels and concurrently increased bone mass.[54] As such, in a mouse model with osteocytes ablated of PTH1R, intermittent PTH failed to suppress SOST expression, with an undetectable effect on bone formation.[56] Similarly, serum sclerostin levels in healthy women are inversely correlated to PTH serum levels, while postmenopausal women treated with teriparatide also demonstrate reduced serum sclerostin levels. In addition to the effects of PTH on sclerostin levels, a large body of work has investigated the role of PTH on canonical Wnt antagonists Dickkopf-1 (Dkk1). Unlike sclerostin, Dkk1 is expressed in cells of the osteoblast lineage, though similarly acts on LRP5/6 to inhibit Wnt signalling.[57] Intermittent PTH has been found to reduce Dkk1 mRNA levels, leading to the functional activation of the canonical Wnt pathway.[58,59] These *in vitro* findings were supported by *in vivo* work on transgenic mice overexpressing Dkk1, which, when dosed with intermittent PTH, demonstrated a blunting of the anabolic effect. There remains some controversy on the importance of Dkk1 specifically, as opposing studies have demonstrated no effect of increased Dkk1 levels on Wnt signalling, with variable results on the actions of PTH.[60,61]

A growing body of work has investigated the effect of PTH on LRP5 and LRP6. Inactivation of LRP5 has been shown to result in osteoporosis-pseudoglioma syndrome[62] associated with premature generalised osteoporosis, while LRP6 mutation leads to early coronary disease in addition to severe osteoporosis.[63] Significantly, intermittent PTH still exerts a bone-forming effect in LRP5-deficient mice, yet, as the PTHR1/LRP6 complex leads to the upregulation of β-catenin signalling, and thus TCF/LEF-mediated stimulation of bone formation,[64,65] LRP6 is specifically required for differentiation and survival of osteoblasts during bone remodelling.[65] Resultantly, the

anabolic effects of intermittent PTH are significantly blunted in LRP6 knockout mice.[63-66]

The activation of Wnt signalling is dependent on a multitude of factors, one of which is osteoimmunity and the role of T cells. In the absence of T cells, it has been demonstrated *in vivo* that intermittent PTH does not induce increased proliferation or differentiation of osteoblasts, nor does it reduce apoptosis. Both CD4 and CD8 T cells are thought to be particularly vital, with *in vivo* studies outlining the ability of CD8 T cells to potentiate intermittent PTH anabolism through its provision of Wnt-10b.[67,68] Similarly, human studies have demonstrated that in the context of teriparatide treatment, again, CD8 T cells were the main source of increased levels of Wnt-10b; this finding was not replicated in patients with primary hyperparathyroidism.[68]

Stromal cell activation and mobilisation. Studies have identified the pivotal action of intermittent PTH on the reactivation of quiescent periosteal lining cells into active osteoblasts, subsequently leading to an increase in overall osteoblast number and net bone formation.[69-71] *In vivo* lineage tracing has demonstrated increased osteoblast number on the periosteal surface of animals treated with intermittent PTH. This is in the context of reduced bone lining cell fraction, reactivated traced lining cells and the absence of increased osteoblast proliferation overall,[70,71] all of which led to significant increases in osteoblast proliferation.

The effect of intermittent PTH on the bone marrow stromal cell niche has also been elucidated, identifying actions on perivascular niches created in part by mesenchymal stromal cells and often located near trabecular bone. Calvi et al[72] outlined the pivotal role of intermittent PTH on the stem cell niche microenvironment, demonstrating the regulatory role of osteoblasts on the haematopoietic stem cell niche using transgenic mice with activated PTH/PTHrP receptors (PPRs). The receptor-specific osteoblasts were found to produce increased levels of the Notch ligand *Jagged 1*, resulting in an increased volume of haematopoietic stem cells with Notch ligand activation. Their further work assessed this effect *in vivo*, whereby intermittent PTH was administered to mice undergoing myeloablative bone marrow transplantation.[73] Results showed a 73% improvement in animal survival after 28 days, with an expansion of bone marrow cellularity and reduced adipocytes when treated with PTH.

The ability of intermittent PTH to mobilise or increase the migration of cells from the haematopoietic niche is particularly significant in the context of sites of increased bone turnover (fractures and peri-implant). Cells are initially 'mobilised' from their niche into the circulation, migrate across the tissue endothelium and mature into active cell types, eventually 'modulating' the local environment. The SDF-1/CXCR4 axis has been found to be an important regulator of stem cell migration.[74-77] Stromal

derived factor-1/CXCL12 (SDF-1) is produced by a multitude of tissue types including fracture endosteum, and in its active form is bound to the chemokine receptor type 4 (CXCR4) r found on mesenchymal stem cells. Granero-Moltó et al[78] demonstrated that dynamic stem cell migration to the fracture site in a stabilised tibial osteotomy model was CXCR4-dependent. The clinical significance of the SDF-1/CXCR4 axis has further been alluded to, whereby the overexpression of CXCR4 on mesenchymal stem cells leads to significant increases in bone mineral density, thus having implications in the treatment of osteoporosis.[78] In addition to a body of work from haematology and cardiologists,[79-81] Kitaori et al[82] demonstrated increased osteoblast expression of SDF-1 following intermittent PTH administration and thus upregulation of the stem cell homing axis SDF-1/CXCR4, with significant implications for endochondral repair and increased bone volume fraction.[82]

Catabolic role of PTH. As discussed, many of the effects of intermittent PTH are mediated via actions on the PTHR1 receptor, affecting both aspects of skeletal remodelling, with net anabolic or catabolic effects dependent upon duration of exposure and dosage. *In vivo* and *in vitro* studies have demonstrated that these resorptive effects are a result of direct and indirect osteoclast activation, and of cascades mediated by both osteoblast and osteocyte actions.

The RANKL/OPG axis

Unlike the multiple mediators of the anabolic actions of intermittent PTH, it is the Receptor activator of nuclear factor kappa-B ligand/osteoprotegerin ligand (RANKL/OPG) pathway that predominantly regulates the effects on osteoclastogenesis of intermittent PTH. RANKL produced by osteoblasts binds to RANK on the surface of osteoclast precursors, leading to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)activation, and ultimately maturation and terminal differentiation of these cells into mature osteoclasts.[83-84] Conversely, OPG secreted by stromal cells is a soluble decoy receptor, binding to RANKL, inhibiting RANK activation and in turn reducing bone resorption.[83-84] The varying balance between resorption and formation, as seen with continuous and intermittent PTH, can in part be explained through the regulation of the RANKL/OPG pathway. The mRNA encoding for RANKL is increased and for OPG decreased in the presence of continuous PTH, leading to increased osteoclastogenesis and thus bone resorption.[85,86] As such, human studies also confirm the effects of PTH exposure on serum RANKL levels, with these markers correlating with femoral bone loss and increased bone resorption markers[87] in patients with primary hyperparathyroidism, demonstrating increased serum RANKL and the RANKL/OPG ratio.[88] In addition, recent work has further highlighted the role of osteocytes whereby transgenic mice, either overexpressing or ablated of PPR/RANKL, demonstrated increased osteocyte production of RANKL following continuous PTH and subsequently had increased bone loss.[89]

Although not yet fully understood, there is physiological coupling between osteoclasts and osteoblasts mediated by systemic hormones.[90] Intermittent PTH mimics this trend, coupling osteoclasts to osteoblasts and highlighting the pertinent role of active resorption. Evidence has demonstrated the direct resorptive effects of PTH, by activation of a PTHR1 receptor identified on osteoclasts, with a number of studies identifying upregulation of the receptor in pathological states.[91,92] Such findings suggest a dual mechanism whereby PTH acts not only directly on osteoclasts but also indirectly via osteoblasts.

Similar to the pro-osteoclast activity of upregulating RANKL levels, PTH has also been shown to affect cell response and production of macrophage colony-stimulating factor (M-CSF). M-CSF is a cytokine involved in the regulation of both cell proliferation and differentiation from the bone marrow niche; *in vivo* stimulation by PTH leads to its release from osteoblasts and subsequent effects on osteoclasts.[93] Indeed, murine studies have demonstrated that increased RANKL and M-CSF levels stimulated osteoclast formation and bone resorption following intermittent PTH treatment, though this effect was ameliorated after 14 days.[94] The importance of increased osteoclast number, through increases in the RANKL/OPG ratio and M-CSF mediated by intermittent PTH, has an unclear role in the overall bone anabolism.[95,96] The relationship between osteoclast and osteoblast number may further underpin bone formation, and, as such, may be integral to coupled bone formation.[88,89]

Monocyte chemoattractant protein-1 (MCP- is one of the key chemokines that regulate migration and infiltration of monocytes or macrophages. MCP-1 is produced by many cell types, including osteoblasts, endothelial cells, fibroblasts, epithelial, smooth muscle cells, mesangial cells, astrocytes, monocytes, and microglial cells. Chemokines selectively recruit monocytes, neutrophils, and lymphocytes, and induce chemotaxis through the activation of G-protein-coupled receptors. MCP-1 specifically plays an active role in PTH-induced bone resorption. Via the cAMP/PKA pathway, *in vitro* studies demonstrated increased expression of MCP-1 in rat osteoblasts following exposure to both intermittent and continuous PTH, leading to bone resorption through chemoattraction of RANKL-activated osteoclastogenesis and pre-osteoclasts. Importantly, although sustained during continuous infusion, intermittent PTH administration led to an initial spike followed by a rapid degradation in MCP-1 levels.[97] As would be expected, patients with primary hyperparathyroidism demonstrated increased serum MCP-1 levels which, like RANKL, fell following parathyroidectomy.[98]
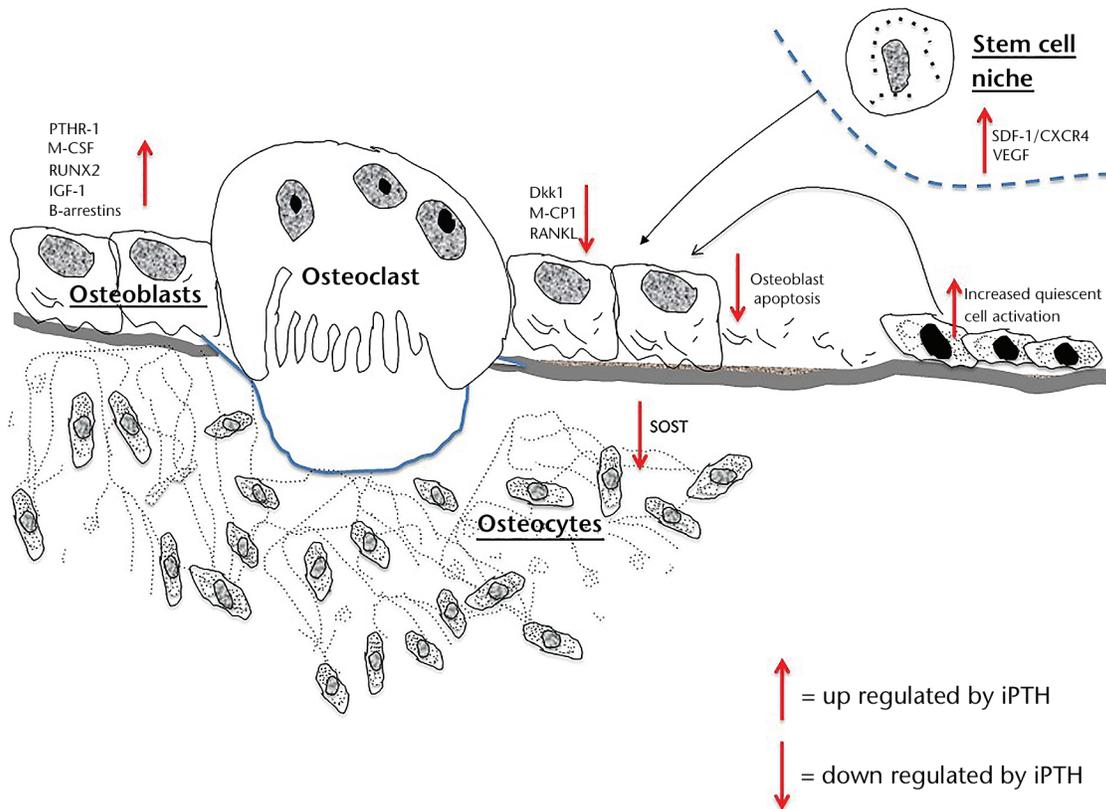
Fig. 1

Illustration of the regulatory actions of intermittent PTH 1-34

Modelling *versus* remodelling. The modalities discussed thus far pertain to a balance of bone formation and resorption; intermittent PTH is known to rapidly increase markers of formation prior to also having an effect on resorption. Yet within this window, as previously discussed, intermittent PTH also activates quiescent lining cells on the modelling surface to further induce bone formation (Fig. 1). This 'modelling' mode of formation was initially identified with the use of double tetracycline labelling in osteoporotic women.[99] Rodent data suggested that modelling accounted for only 20% of bone formation, with this figure further decreasing with age, while human studies indicate between 5% and 30% of formation occurs at modelling surfaces.[42,100] Rhee et al[100] further demonstrated the role of intermittent PTH on modelling-based bone formation, whereby RANK deficient mice were treated with intermittent PTH, and thus remodelling was ablated in the absence of active osteoclasts. Resultantly, short-term high-dose PTH led to increases in serum osteocalcin, and trabecular and cortical bone mineral density. More recent evidence has suggested that the effect of intermittent PTH on modelling is most significant in the early stages of treatment in human populations, whereby the proportion of modelling-based bone formation reduces in the steady state situation following long-term intermittent PTH treatment in osteoporotic populations.[101,102]

In both modelling- and remodelling-based bone formation, the actions of PTH receptor signalling on osteocytes are pivotal. DMP1–8kb-caPTHR1 transgenic mice express a constitutively active PTH osteocyte receptor and, in conjunction with anti-resorptive agents, have been used to investigate the role of modelling and remodelling within various bony compartments.[100] Subsequently, on the periosteal cortical surface, inhibiting remodelling-based bone formation had no effect on overall bone formation or bone mineral density. Importantly, when SOST was overexpressed and thus the Wnt pathway blocked, this modelling-based bone formation at the periosteal surface was ablated.[54] Ultimately, in animal studies, bone formation induced by osteocytic PTH receptor signalling on the periosteal surface appears to be Wnt pathway-dependent and independent of bone resorption; as such, periosteal bone formation enhanced by intermittent PTH may be predominantly a result of modelling effects.

Recently, increased investigation into PTHR1 activation has led to the development of abaloparatide, a synthetic analogue of PTHrP 1-34, which has demonstrated a lower catabolic profile than teriparatide, and thus clinically would have fewer hypercalcaemia-related side effects.[99] Work has demonstrated that structurally distinct ligands can bind to differing receptor conformations; the

R0 receptor conformation is G-protein-independent and thus unaffected by analogues that act to dissociate G protein-receptor complexes. Consequently, when activated with long-acting ligands like long-acting -PTH, the net effect is an increase in catabolism and net resorption. Conversely, abaloparatide and short-acting ligands have been found to have an affinity for the G-protein-sensitive receptor conformation RG.[103-105] Subsequently, activation of the receptor occurs only briefly due to rapid dissociation of the ligand receptor complex upon G protein activation, thus inducing a net anabolic response *in vivo*. Phase 2 trials suggest that abaloparatide has a smaller stimulatory effect on remodelling than does teriparatide. As such, in clinical osteoporotic populations, this analogue may lead to a reduction in non-vertebral fractures with a concurrent improvement in cortical porosity and bone strength. Importantly, PTH 1-34 and 1-84 both led to abnormal bone architecture in murine studies, with a reported incidence of osteosarcomas following lifetime treatment,[106-108] though this has not been reported with the use of abaloparatide.

Parathyroid hormone has profound and complex effects on the skeleton; its elevation in the circulation can generate both catabolic and anabolic effects depending on the temporal profile of its increase, and, as such, is used clinically in short bursts (12- to 18-month cycles). At the cellular level, intermittent PTH directly stimulates bone formation via osteoblasts, increasing number and activity; concurrently, intermittent PTH stimulates bone resorption by also increasing the recruitment and activation of osteoclasts. In conclusion, crosstalk between modelling- and remodelling-based bone formation is driven by PTH receptor signalling in osteocytes, osteoclasts, osteoblasts and undifferentiated cells. Ultimately there is no 'common' intermittent PTH pathway; instead, the hormone acts via multiple mechanisms to exert its anabolic effect. By understanding both the anabolic and catabolic actions of PTH 1-34, one can hope to enhance its clinical utility as a mode of increasing bone formation in both the osteoporotic and fracture-healing contexts.

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