

## PERSPECTIVES

### Insulin-like Growth Factor-I and Bone

Daniel D. Bikle and Yongmei Wang

*Veterans Affairs Medical Center and University of California San Francisco, San Francisco, California, USA*

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#### Abstract

Insulin-like growth factor-I (IGF-I) is produced by chondrocytes, osteoblasts, osteocytes, and osteoclasts, and its receptor (IGF-IR) is found in all of these cells. The actions of IGF-I are mediated by IGF-IR, a tetrameric membrane-bound receptor homologous to the insulin receptor. When activated this receptor sets into motion two signaling pathways, the PI3K/Akt pathway and the Ras/Raf/MAPK/ERK pathway. Skeletal loading *in vivo* or of osteoblasts and osteocytes *in vitro* enhances IGF-I signaling by inducing IGF-I production and stimulating the interaction between IGF-IR and selected integrins that are critical for IGF-I signaling. The actions of IGF-I are regulated by the six IGF-binding proteins in both an inhibitory and stimulatory fashion. Different binding proteins have different actions often depending on the context of the experimental situation. The relative contributions of circulating IGF-I (produced primarily in the liver) and that produced by bone remain in dispute, although both are involved in skeletal development. Cell-specific deletion of IGF-IR blunts chondrocyte and osteoblast proliferation and differentiation and inhibits osteoclastogenesis. IGF-I facilitates the skeletal actions of a number of calciotropic hormones including parathyroid hormone (PTH), parathyroid hormone-related protein (PTHrP), growth hormone (GH), thyroid hormone, and glucocorticoids, and regulates the production of PTHrP and GH. Thus through numerous means IGF-I plays a key role in all aspects of skeletal development, the skeleton's subsequent remodeling, and its response to hormonal and environmental factors. This *Perspective* focuses on the role of IGF-I and IGF-IR in the regulation of skeletal growth and remodeling. *IBMS BoneKEy*. 2011 July;8(7):328-341.

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#### Introduction

Insulin-like growth factor-I (IGF-I), acting through its receptor (IGF-IR), plays a critical role in all aspects of skeletal development and bone remodeling. Indeed, all cells in the skeleton produce IGF-I, and respond to it via IGF-IR. This *Perspective* discusses the pathways mediating IGF-I signaling in cells, including the role of integrins in regulating IGF-I responsiveness. The impact of the different IGF-binding proteins on IGF-I will also be examined, as will findings from studies of genetically altered mice in which IGF-I and IGF-IR have been overexpressed or deleted globally or in specific bone cells. Data addressing the question of the relative contributions of systemic and paracrine production of IGF-I to skeletal development and growth will also be examined. Finally, this article reviews the role that IGF-I

signaling plays in the skeletal actions of parathyroid hormone (PTH), parathyroid hormone-related protein (PTHrP), growth hormone (GH), thyroid hormone, and glucocorticoids. All of this research makes clear that the role of IGF-I should be considered in all studies concerning the skeleton.

#### Mechanisms of Action of IGF-I on Bone

##### *IGF-I signaling pathways*

IGF-I acts through IGF-IR, which consists of two  $\alpha$  and two  $\beta$  subunits (Fig. 1) (reviewed in (1)). The binding of IGF-I to IGF-IR results in activation of its intrinsic tyrosine kinase. The kinase domain resides within amino acids 956-1256; activation entails the sequential tyrosine phosphorylation of residues Y1135, Y1131, and Y1136, which

alters the structure of the  $\beta$  chain, enabling its kinase activity to be expressed (2). Mutation of these tyrosines to phenylalanine also impairs the ability of IGF-IR to complex

with other signaling molecules, including specific integrins (3).

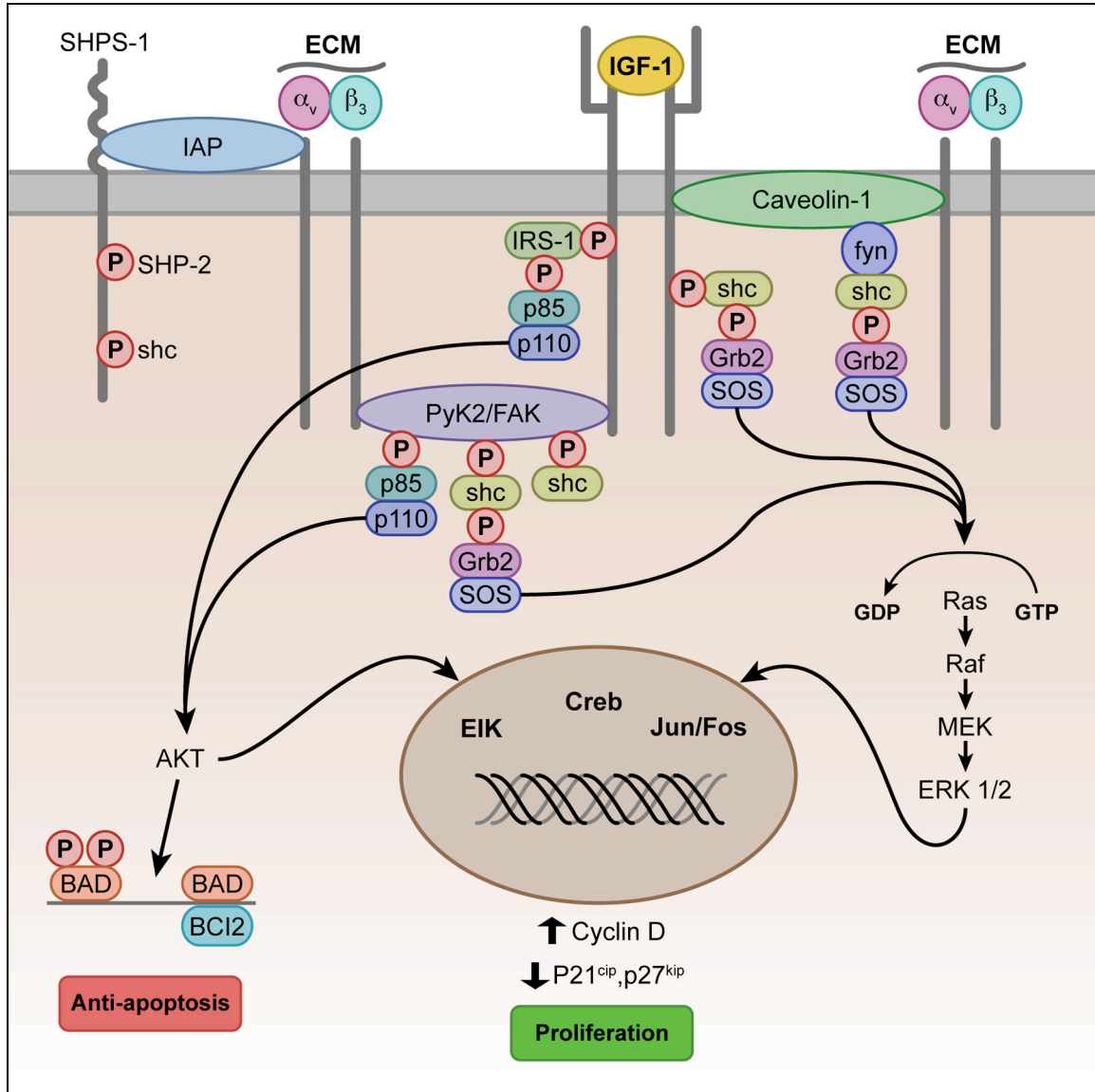


Fig. 1. Working model of IGF-IR/integrin interactions in bone cells. IGF-IR forms a complex with  $\alpha\beta_3$  integrin that is required for IGF-I activation of IGF-IR. Mechanical load increases whereas unloading decreases formation of this complex and thus regulates IGF-I responsiveness. Formation of the integrin/IGF-IR complex brings to IGF-IR non-receptor kinases such as FAK family and src family kinases that are hypothesized to activate IGF-IR independently and/or synergistically with IGF-I. Caveolin may also contribute to the formation of an integrin IGF-IR complex. SHPS-1/SHP-2 has been shown to play a role in regulating IGF-I signaling in some tissues, but this role in bone cells is unclear. The IGF-IR signals through two main pathways involving PI3K/Akt and Ras/Raf/MEK/ERK, respectively. Akt, by phosphorylating BAD, has an anti-apoptotic effect, while ERK promotes proliferation. However, these represent just two of a number of intracellular events triggered by the activated IGF-IR. In either case, IRS-1 and -2 help mediate these downstream actions of IGF-IR.

These and subsequent phosphorylations create multiple docking sites for a variety of

endogenous substrates including members of the insulin receptor substrate (IRS) family

that associate with IGF-IR via PTB and SH2 domains, growth receptor binding protein-2 (Grb2) that binds to specific motifs in IGF-IR as well as in IRS, and the p85 subunit of phosphatidylinositol 3 kinase (PI3K), which binds to other specific motifs within IRS. Both IRS-1 and IRS-2 are expressed in bone (4;5) and both appear to be required for the full actions of IGF-I, but their roles differ. Deletion of IRS-1 results in decreased bone formation and resorption (4), whereas deletion of IRS-2 results in reduced bone formation but increased bone resorption (5). Their exact means of mediating IGF-I signaling remains unclear. Shc, when tyrosine phosphorylated in response to IGF-I, binds to the SH2 domain of Grb2, which in turn forms a complex with Sos, a guanine nucleotide exchange factor that mediates GDP/GTP exchange in ras and thus activates it. Ras then activates Raf (MAPKKK), which phosphorylates and activates MEK (MAPKK), which in turn phosphorylates and activates ERK1/2 (MAPK); these are serine/threonine phosphorylations. Activated ERK enters the nucleus to phosphorylate and thus activate transcription factors (e.g., elk-1 and c-jun) leading to increased cyclin D<sub>1</sub> and reduced p21<sup>cip</sup> and p27<sup>kip</sup> expression. The increased levels of cyclin D<sub>1</sub> and reduced levels of the cell cycle inhibitors p21<sup>cip</sup> and p27<sup>kip</sup> stimulate cell cycle progression from G1 to S, thus completing the pathway by which IGF-I and other growth factors promote proliferation.

Activation of PI3K sets up a different pathway. PI3K phosphorylates PIP<sub>2</sub> to PIP<sub>3</sub> in the membrane, recruiting Akt to the membrane where it is phosphorylated and activated by PDK1/2. The activated Akt then phosphorylates and inactivates Bad, a proapoptotic member of the bcl-2 family. This pathway blocks apoptosis. In addition, Akt increases protein synthesis and via its actions on mTOR and p70S6 kinase (6) and may stabilize  $\beta$ -catenin (and thus promote proliferation) by inhibiting its phosphorylation by GSK-3 $\beta$  (7). Hypoxia-inducible factor (HIF)- $\alpha$  is also induced by IGF-I through the PI3K/Akt/mTOR pathway, leading to increased VEGF-A production and vascular invasion in the growth plate (8). Moreover, PI3K and Akt can enter the nucleus and by

phosphorylating critical transcription factors also lead to increased cyclin D1 levels.

#### *The role of integrins in regulating IGF-I responsiveness in bone*

The role of integrins in regulating IGF-I responsiveness has been demonstrated in a number of tissues (Fig. 1). Perhaps the most extensive examination has come from the Clemmons laboratory that has studied this phenomenon in aortic smooth muscle cells (9-11). These investigators have found that echistatin (a disintegrin) or blocking antibodies to the integrin  $\alpha$ v $\beta$ 3 block IGF-I-stimulated proliferation, IGF-I receptor autophosphorylation, IRS-1 phosphorylation, and binding of the p85 subunit of PI3K to IRS-1. Their proposed mechanism is that integrin activation recruits the tyrosine phosphatase SHP-2 to the  $\beta$ 3 integrin subunit. In turn, IGF-I, via IGF-IR, phosphorylates and thus activates the transmembrane protein SHPS-1, which recruits SHP-2 to SHPS-1 for an as yet undefined role in further signaling. When  $\alpha$ v $\beta$ 3 activation is blocked, SHP-2 is instead recruited to IGF-IR where it dephosphorylates and thus terminates the activation of IGF-IR. Results in bone cells from studies by our group indicate a different mechanism. We have shown that although echistatin blocks IGF-I activation of IGF-IR, neither skeletal unloading nor echistatin alters the recruitment of SHP-2 to IGF-IR nor the timing of IGF-IR phosphorylation and dephosphorylation (12). Instead, IGF-IR is just not phosphorylated in response to IGF-I in bone marrow stromal cells (BMSCs) from unloaded bone or in BMSCs from normally loaded bones treated with echistatin, and the downstream MAPK and PI3K/Akt pathways are not activated. IGF-I responsiveness in bone cells appears to require direct binding of  $\alpha$ v $\beta$ 3 to IGF-IR (13). The matrix protein vitronectin is a potent stimulator of  $\beta$ 3 integrin subunit phosphorylation, and potentiates the ability of IGF-I to do likewise (11;14). Mutation of the tyrosines involved (amino acids 773, 785) in the  $\beta$ 3 integrin subunit blocks IGF-I signaling (11;14). Moreover, IGF-I has been shown to bind  $\alpha$ v $\beta$ 3 integrin directly, and when IGF-I is mutated (R36E/R37E) it fails to bind  $\alpha$ v $\beta$ 3 while retaining its affinity for

IGF-IR, but is unable to activate IGF-IR (15). Skeletal unloading results in decreased expression of  $\alpha v\beta 3$  (12). Kapur *et al.* (16) demonstrated that fluid shear stress on bone cells stimulates IGF-IR phosphorylation independently of IGF-I, that this response is potentiated by fibronectin and vitronectin, and that the response is blocked by echistatin. Thus, mechanical load increases bone formation in part by stimulating formation of an IGF-IR/integrin complex, sensitizing the osteoblast to the anabolic actions of IGF-I.

### Regulation of IGF Action by IGF-Binding Proteins

Osteoprogenitor cells express all six of the high affinity IGF-binding proteins (IGFBPs or BPs), and chondrocytes express all but BP-1 (17). The expression level of these BPs changes with differentiation of the cells (17). The impact of these BPs on the action of IGF on bone is quite complex, varying according to the BP being evaluated, the molar ratio with IGF, the type and differentiation level of the cells being evaluated, the effect of matrix, post-transcriptional modification, and proteolytic processing (17). In general, all BPs, by binding IGF, have the potential to inhibit IGF action. However, BPs have effects independent of IGF (17), likely contributing to the substantial differences that exist among the different BPs with respect to their skeletal actions. Transgenic overexpression of BP-2 leads to skeletal deficiency (18), but in equimolar concentrations with IGF-II BP-2 can promote rat osteoblast differentiation (19). This is thought to be due to its binding to the matrix and increasing IGF availability to the osteoblast (20). The rare syndrome of osteosclerosis in patients with hepatitis C is associated with increased BP-2 (20), perhaps by this mechanism. BP-2 null mice show gender-specific differences in that female null mice have increased cortical bone, whereas the males are osteopenic (21). Transgenic overexpression of BP-3 also leads to a reduction in bone, due both to an increase in osteoclastogenesis and a decrease in osteoblast proliferation (22). However, in combination with equimolar concentrations of IGF-I, BP-3/IGF-I

increased bone mass in ovariectomized rats (23). Transgenic overexpression of BP-4 decreased osteoblast number and trabecular bone (24). However, proteases such as pregnancy-associated plasma protein A (PAPP-A) are also expressed in bone and by cleaving BP-4 can release the bound IGF, promoting bone formation (25). BP-5 is the most abundant BP in both bone and cartilage (17). Transgenic overexpression of BP-5 results in a gender-specific (males more than females) and site-specific (periosteal, not endosteal) reduction in bone formation (26;27), although subcutaneous injection of BP-5 over bone increased trabecular bone formation in ovariectomized mice (28). This may be due to the ability of BP-5 to bind to matrix, which decreases its affinity for IGF, thus releasing IGF in the vicinity of osteoblasts (29). On the other hand, differences between overexpression of a BP and its administration with IGF may indicate the IGF-independent action of these molecules (17). BP-1 and -6 have received less study in bone. PTH stimulation of BP-5 expression has been observed (30), but this has not been reported for other BPs.

### Lessons from Transgenic Mice

Mice lacking the ability to produce IGF-I (IGF-I KO) are born small (approximately 60% of normal weight) and, if they survive the birth process, fail to grow normally such that by 8 weeks of life they are 30% of normal weight (31-33). Mineralization of the axial skeleton and calvarium is delayed in the embryo, and reduced chondrocyte proliferation and differentiation with increased chondrocyte apoptosis are found. The Indian hedgehog (Ihh)/PTHrP loop in the growth plate is disrupted with reduced Ihh expression in pre-hypertrophic cells but increased PTHrP expression (34). A similar phenotype was found in a human born with an IGF-I deletion (35). Mice lacking IGF-IR globally do not survive. Bone formation is reduced in the adult IGF-I KO, but trabecular bone volume (BV/TV) in the proximal tibia is increased (31), a result reflecting the dual effect of IGF-I on osteoblast and osteoclast activity (36). Colony-forming units are reduced in BMSC cultures from these mice.

Although the anabolic action of IGF-I is well-appreciated, IGF-I is also catabolic. This is indicated by the decrease in BV/TV and increased osteoclast numbers especially in the calvaria of mice over-expressing IGF-I under the control of the collagen  $1\alpha 1$  promoter (37;38), although an earlier study in which IGF-I was overexpressed in osteoblasts using an osteocalcin promoter showed an increase in BV/TV at six weeks (but not at 3 or 24 weeks) (39). Osteoclasts express IGF-IR (40;41), produce IGF-I themselves (42;43), and IGF-I stimulates RANKL expression and osteoclastogenesis in BMSCs (36;44;45). On the other hand, global IGF-I KO have low levels of expression of RANKL as well as deficient ephrin B2 and EphB4 (46), two recently discovered molecules mediating bi-directional signaling between osteoblasts and osteoclasts (47) and between osteoblasts themselves (48). Deleting IGF-IR from osteoclast precursors *in vitro* blocks osteoclastogenesis (36). Osteoblasts from IGF-I KO have decreased expression of RANKL and are poor stimulators of osteoclastogenesis (36); this can be reversed with the addition of IGF-I. Thus, both osteoblasts and osteoclasts are producers and targets of IGF-I.

To distinguish which cells are responsible for the IGF-I KO phenotype and its lack of responsiveness to PTH, we and others have selectively deleted IGF-I and IGF-IR from chondrocytes, osteoclast precursors and osteoblasts of different maturities. Deletion of IGF-IR specifically in chondrocytes by breeding mice with a floxed IGF-IR with mice expressing cre recombinase under a collagen II promoter (IGF-IRcart KO) resulted in mice with a phenotype quite similar to that described above for global IGF-I deletion (49). Postnatal survival is limited and growth reduced although not as severely as in the global IGF-I KO (90% of wild-type at birth). Mineralization of the axial skeleton and calvarium is delayed, the chondrocytes show decreased proliferation and increased apoptosis, and expression of PTHrP is increased (49). To evaluate the impact of IGF-IR deletion in chondrocytes postnatally, we bred the floxed IGF-IR mice with mice in which the collagen II promoter

was regulated by tamoxifen, and injected the mice and their cre-negative littermates postnatally with tamoxifen starting at 1 week (tamIGF-IRcart KO) (49). Prior to the start of tamoxifen injections all mice were normal in size. By two weeks the tamIGF-IRcart KO were 70% the size of the control mice. As in the IGF-IRcart KO, proliferation of the chondrocytes was markedly impaired, and the hypertrophic zone was reduced with increased PTHrP expression. When IGF-I from chondrocytes was deleted using a similar approach by Govoni *et al.* (50), only a 40% reduction in IGF-I expression was observed in cartilage, with no apparent impact on postnatal survival or body size at birth, but with a 25% reduction in growth and total bone mineral content over the 2-4-week period. No histomorphometry was performed. These differences from our own experience may reflect the limited efficiency of the cre recombinase utilized by Govoni *et al.* (50). When IGF-IR from osteoclasts is selectively deleted using a TRAP5b-driven cre recombinase (IGF-IRoc KO), BV/TV is increased, consistent with decreased numbers of osteoclasts (51). When IGF-IR is selectively deleted from mature osteoblasts using an osteocalcin (OCN)-driven cre recombinase (IGF-IRob KO), bone mineralization is reduced, despite normal numbers of osteoblasts (52). Although the number of colony-forming units in BMSC cultures from such mice is normal, the colonies fail to mineralize (53). In contrast, when IGF-IR is deleted from osteoprogenitors using an osterix-driven cre recombinase (IGF-IRop KO), osteoblast numbers, proliferation, and differentiation are all reduced, resulting in decreased BV/TV (54). In contrast to our studies in which postnatal survival and growth are well-maintained in the osteoblast-specific knockouts, Govoni *et al.* (55), using a collagen  $1\alpha 2$ -driven cre recombinase to delete IGF-I from osteoblasts, observed high perinatal mortality and poor growth, with little change in BV/TV in cancellous bone. However, this cre recombinase is not specific for bone, but was found at high levels in muscle and other non-skeletal tissues (55). Deletion models of IGF and IGF-IR influence on PTH action are shown in Table 1.

Table 1. Deletion models of IGF and IGF-IR influence on PTH action

Model	Impact on bone	Reference
Global IGF-I KO	↓ BFR ↑ BV/TV ↓ OC ↓ Growth, poor survival ↓ Chondrocyte proliferation ↑ Chondrocyte apoptosis ↓ Chondrocyte differentiation	(31) (34) (32) (33) (36)
Mature OB-specific IGF-IR KO	↓ Mineralization Normal OB	(71) (52)
Immature OB	↓ OB ↓ BV/TV ↓ Mineralization ↓ Differentiation	(54) (55)
OC-specific IGF-IR KO	↓ OC ↑ BV/TV	(36) (51)
Chondrocyte-specific IGF-IR KO	Similar to growth plate abnormalities in global IGF-I KO	(49) (50)
Immature OB-specific IGF-I overexpresser	↓ BV/TV	(38)
Mature OB-specific IGF-I overexpresser	↑ BV/TV	(39)

KO: knockout; BFR: bone formation rate; BV/TV: bone volume/total volume; OC: osteoclast; OB: osteoblast

### Relative Contributions of Systemic and Locally Produced IGF-I

The liver is the major source of circulating IGF-I, where its production is controlled primarily by GH. Liver-specific deletion of IGF-I results in a 75% reduction in circulating IGF-I with little or no change in body growth or bone length. When combined with global deletions of the acid labile subunit (ALS) and IGF-BP-3 (triple KO) to reduce circulating IGF-I levels by 97.5%, body length was reduced by 6% and cortical bone width and BV/TV by 18% and 8%, respectively (56;57). This finding, however, needs to be compared to the substantially greater impact of the global IGF-I KO described above. Complicating the interpretation, however, is the three-fold increase in GH in the liver-specific IGF-I KO and the six-fold increase in the triple KO (56). Liu *et al.* (58) have shown that although global IGF-I KO fail to respond to GH, mice lacking IGF-I production only in the liver do respond. Thus, this increase in GH would be expected to increase skeletal production of IGF-I, thus providing some compensation for the fall in circulating IGF-I. In addition, global deletions of ALS and IGF-BP-3 impose their own specific impact on bone independently of their roles in maintaining circulating levels of IGF-I (56). A

second approach to this question involves rescue-type experiments in which IGF-I production is restored specifically to the liver in mice with the global IGF-I null background. In one study, Stratikopoulos *et al.* used an elegant knock-in strategy to selectively turn on IGF-I production in the liver (59). They restored serum levels of IGF-I to 50% of normal and increased the body weight of the mice from 34% of normal in IGF-I KO to 50% of normal in their “rescued” mice. No evaluation of the bones was performed. Elis *et al.* developed a liver-specific transgenic mouse in a global IGF-I null background and increased serum IGF-I levels to two- to three-fold above wild-type control mice (60). These mice exhibited growth and most skeletal parameters comparable to wild-type controls, although not to the degree seen in transgenic mice achieving a comparable level of IGF-I in a wild-type background. Thus it would appear that local production of IGF-I by the skeleton is important for bone development and growth, but supraphysiologic levels of circulating IGF-I can compensate. However, Elis *et al.* did not demonstrate that their transgene was expressed only in the liver and not also in bone, an omission that must temper this conclusion (60).

### The Role of IGF-I in the Skeletal Actions of Systemic Hormones

The skeletal production of IGF-I postnatally is controlled primarily by GH (61) and PTH (62;63). Regulation of IGF-I expression during embryologic development is unclear, although PTHrP is a good candidate. GH receptors (GHR) have been demonstrated in chondrocytes (64) and osteoblasts (64), where GH's growth-promoting actions are mediated primarily but not exclusively by IGF-I (66;67). This is well-demonstrated by the ability of IGF-I antibodies to block the growth-promoting actions of GH when injected into bone (66), the markedly stunted growth of IGF-I or IGF-IR null animals despite the presumed elevated circulating GH (67), and the failure of GH to stimulate growth in IGF-I null animals (58). However, we did observe a modest increase in periosteal bone formation rate (BFR) when GH was infused into global IGF-I null mice (68), and Lupu *et al.* found that mice lacking

both GHR and IGF-I were more profoundly growth-retarded than either KO alone (67).

The receptor for PTH and PTHrP is also found in chondrocytes and osteoblasts (69;70), and like GH, many of the skeletal actions of PTH and PTHrP are mediated by IGF-I (71;72). In chondrocytes, antibodies to IGF-I block the ability of PTH and PTHrP to induce aggrecan production (72). In bone, PTH fails to stimulate periosteal BFR in global IGF-I KO (71) or to increase the number of osteoprogenitors in mice lacking IGF-IR specifically in osteoblasts (71). In addition, IGF-I signaling is required for PTH to induce RANKL (71) and ephrin B2/EphB4 (46), potentially affecting PTH stimulation of osteoclastogenesis as well as osteoblast proliferation and differentiation. Our working models for the mechanisms by which IGF-I signaling affects PTHrP signaling in the growth plate and PTH signaling in bone are shown in Fig. 2 and Fig. 3.

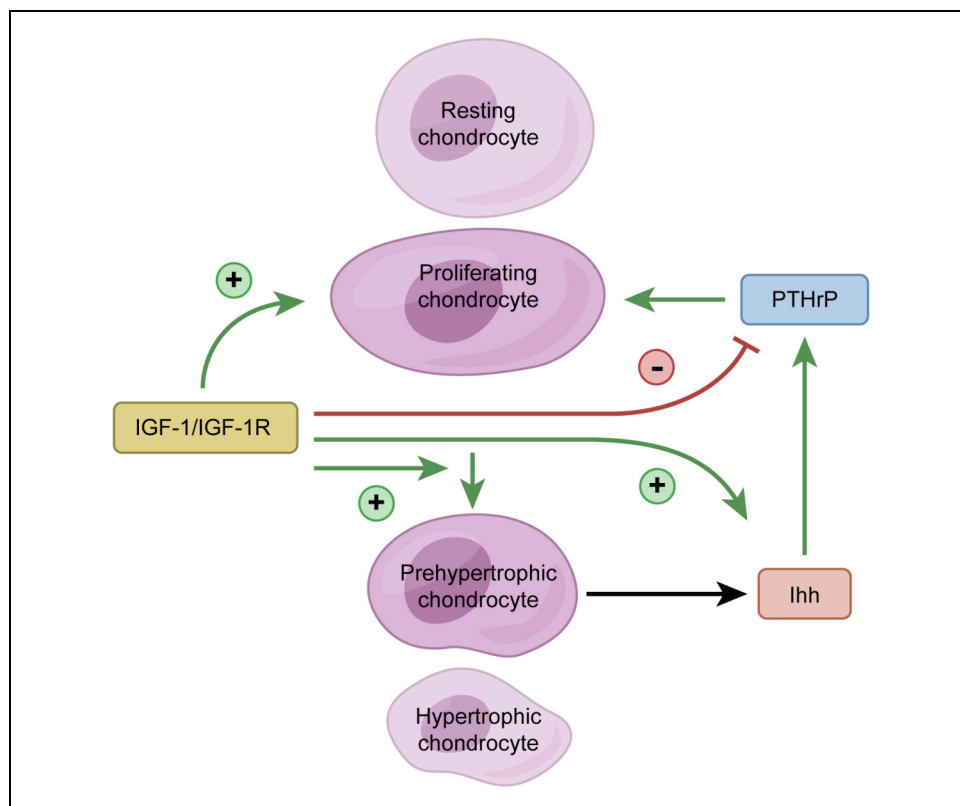


Fig. 2. The role of IGF-I in the actions of PTHrP in the growth plate. IGF-I signaling in the growth plate promotes chondrocyte proliferation and maturation. This pathway promotes cell differentiation at least in part by down-regulating PTHrP expression and up-regulating Ihh expression while mediating the stimulation of chondrocyte proliferation by PTHrP.

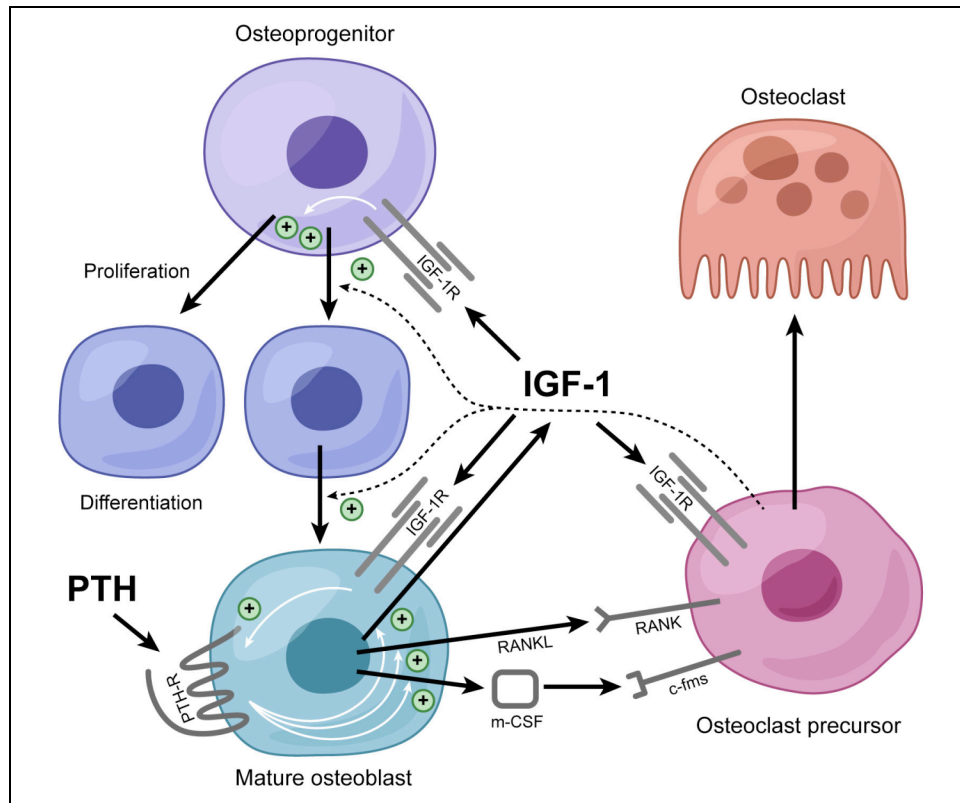


Fig. 3. The role of IGF-1 in the actions of PTH in bone. We propose that in bone, the mature osteoblast is the major responder to PTH and producer of IGF-1. The IGF-1 induced in the mature osteoblast by PTH stimulates the proliferation and differentiation of osteoprogenitors. IGF-1 thus produced also feeds back on the mature osteoblast to enable PTH to induce RANKL and m-CSF that, along with IGF-1, promote osteoclastogenesis.

Thyroid hormone also stimulates the expression of both IGF-1 and its receptor in chondrocytes and osteoblasts (73-76), and this expression underlies at least some of the skeletal actions of thyroid hormone (76). One intriguing concept is that this interaction between thyroid hormone and IGF-1 involves the stabilization of  $\beta$ -catenin via activation of Akt (75), as described earlier. Glucocorticoids, on the other hand, reduce IGF-1 expression in growth plates (77), perhaps contributing to their negative impact on skeletal growth.

### Summary

IGF-1 signaling plays a critical role in all aspects of skeletal development and remodeling. It promotes the proliferation, differentiation, and function of chondrocytes, osteoblasts, and osteoclasts. The major signaling pathways for IGF-1 are mediated by IGF-IR, which, when activated by IGF-1, initiates both the MAPK and PI3K pathways.

Integrins regulate IGF-IR activation, accounting for at least part of the anabolic actions of mechanical load. IGF-binding proteins, by either enhancing or preventing access of IGF-1 to its receptor, further regulate IGF-1 signaling. Both circulating and locally produced IGF-1 participate in its skeletal actions. The skeletal actions of a number of hormones, most particularly GH and PTH, are mediated at least in part through their ability to induce IGF-1 in the growth plate and in osteoblasts. Therefore, when evaluating environmental or hormonal manipulations that impact bone, the role that IGF-1 signaling plays must be considered.

**Conflict of Interest:** None reported.

**Peer Review:** This article has been peer-reviewed.

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