REVIEW

GH/IGF-I and bone resorption *in vivo* and *in vitro*

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Abstract

IGF-I may act as one of several coupling agents by activating bone formation and bone resorption. *In vivo* studies in normal subjects, postmenopausal women and patients with excess or diminished GH production (acromegaly and GHD) indicate that both GH and IGF-I activate osteoclasts, but that GH has a more pronounced effect, independently of IGF-I. *In vitro*, GH and IGF receptors have been demonstrated on osteoclasts and both GH and IGF-I may directly modify osteoclast function and activity. In addition to direct effects on osteoclasts, GH and IGF-I may affect bone resorption indirectly by stimulating release of paracrine mediators that regulate osteoclastic resorption (cytokines). Critical for the bone resorptive process is the balance between OPG and RANKL, which is regulated by many systemic factors. *In vivo* and *in vitro*, GH/IGF-I may modulate this balance but these studies are difficult to interpret, reflecting the complexity of this system. Increased OPG expression may possibly protect against GH/IGF-I-induced bone resorption and potentially be important for the long-term beneficial effects of GH replacement. Further studies investigating the OPG/RANKL ratio and system in experimental and transgenic GH/IGF models may clarify these issues.

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Introduction

Growth hormone (GH) plays a crucial role in the maintenance of bone mass in adults by regulating bone remodeling through a complex interaction of circulating GH, insulin-like growth factors (IGFs), IGF-binding protein (IGFBPs) and locally produced IGFs and IGFBPs, acting in an autocrine and paracrine way. Aging is associated with a decline in trabecular and cortical bone mass and with a deterioration of microarchitecture in both skeletal compartments. Ultimately, the predominant cause of bone loss in postmenopausal women is increased bone resorption without a corresponding increase in bone formation. Serum levels of GH and IGFs also decline with increasing age (1–3) and a dysfunctional GH–IGF axis may thus play a role in the pathogenesis of postmenopausal osteoporosis. Thus, GH or IGF-I substitution may be considered a treatment option in these patients. While the effects of GH and IGFs on osteoblastic development and function are well documented, studies on effects of GH and IGFs on bone resorption are relatively scarce. When GH and IGF-I stimulate bone formation, they enhance bone turnover, thereby releasing molecules from activated marrow stromal cells and osteoblasts that also lead to enhanced osteoclastogenesis and mature osteoclast activity. These effects may limit the therapeutic potential of these agents and research

aimed at understanding the effects of GH and IGF on osteoclastic resorption may allow the development of better treatment modalities.

In vivo observations in healthy controls and patients with osteoporosis

Short-term treatment with GH dose-dependently increases biochemical markers of bone resorption in osteopenic postmenopausal women and elderly people (4, 5), and these changes are closely correlated with changes in serum IGF-I, suggesting that GH increases osteoclastic activity possibly through increased systemic and/or local IGF-I (5). However, the effects of GH on bone resorption (and especially bone formation) are blunted in the presence of estrogen in healthy elderly women (6), possibly because estrogen leads to a relative resistance to the stimulatory effect of GH on IGF-I production or antagonism between estrogen and GH at the peripheral tissue level as observed in GH deficiency (GHD) (7). Ebeling *et al.* (8) demonstrated that treatment of normal women with IGF-I activates both osteoclasts and osteoblasts, but with a more prominent effect on bone formation rather than resorption. Other authors have found similar results, showing that IGF-I to a greater degree stimulates osteoblasts selectively (9, 10) and one study found that low

doses of IGF-I had no effect on bone resorption markers during short-term treatment of elderly women (11). Together, these *in vivo* data indicate that both GH and IGF-I activate osteoclasts, but that GH has a more pronounced effect, independently of IGF-I.

***In vivo* observations in patients with GH/IGF-I deficiency and excess**

Important information on the effects of GH and IGFs on bone resorption can be investigated *in vivo* in patient populations characterized by systemic excess or deficiency and the treatment of these. Acromegalic patients have a chronic systemic GH and IGF-I excess and are characterized by increased bone turnover, suggesting activation of both osteoblasts and osteoclasts. Furthermore, biochemical markers of bone formation and bone resorption correlate with circulating GH and IGF-I levels, suggesting that systemic GH and liver-derived IGF-I may have direct effects on both cell types in modulating turnover (12–17). Moreover, normalization of GH/IGF-I levels in these patients during treatment is associated with rapid reductions in turnover markers (16–19). The effect of excess systemic GH in adult transgenic mice is in accord with findings in these subjects, showing a markedly enhanced bone formation followed by an exaggerated bone resorption, resulting in cortical tissue with inferior mechanical properties (20). In contrast, patients with GHD have secondary osteoporosis characterized by reduced bone mass (21–24), decreased bone turnover measured by biochemical markers, and increased fracture risk (24–26). Treatment of GHD patients with GH dose-dependently increases bone turnover and these changes are positively correlated with increases in serum IGFs, as well as GH and IGF-I, suggesting that the GH may increase bone mass partly through changes in systemic levels of IGF family members (27). Furthermore, enhanced cortical bone protein and gene expression of IGF-I is found during GH therapy of adult-onset GHD (aoGHD) adults and these changes are correlated with changes in bone matrix gene expression of calcitonin receptor as well as serum biochemical bone markers, indicating direct effects of locally produced IGF-I on osteoclasts and in regulating bone turnover (28, 29). Similar effects are found in GH-deficient transgenic mice treated with IGF-I (30).

Effects on osteoclasts *in vitro*

In vitro GH and IGFBP-5 stimulate osteoclastic bone resorption through direct and indirect actions on osteoclast differentiation and through indirect activation of mature osteoclasts, possibly via local IGF-I/II production from osteoblasts (31, 32). IGFR1 expression has been demonstrated on mature rabbit osteoclasts, as well as human preosteoclasts (33, 34), and IGF-I

enhances formation of osteoclast-like cells in long-term bone marrow cultures (35, 36). In contrast, IGF-I has an inhibitory effect on stimulated bone resorption in bone organ cultures (36). It is believed that IGF-I/II is incorporated into bone matrix through binding to IGFBP-5 and hydroxyapatite for later release during osteoclastic bone resorption. Thus, IGF-I may act as one of several coupling agents by activating bone formation and bone resorption and the amount of IGF-I released from bone matrix should activate a proportionate response from osteoblasts to produce enough osteoid to fill the resorption lacunae.

Effects of GH/IGF-I on immune cells and cytokines

In addition to direct effects on osteoclasts, GH and IGF-I may affect bone resorption indirectly by stimulating release of paracrine mediators that regulate osteoclastic resorption. Experimental and clinical studies implicate members of the interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)- α superfamilies in the pathogenesis of postmenopausal osteoporosis. Increased levels of these mediators are associated with enhanced osteoclastogenesis and a negative remodeling balance (37–39). *In vitro*, both GH and IGF-I increase mouse and human osteoblastic production of IL-6 (40, 41). Furthermore, experimental models show that enhanced T cell production of TNF resulting from increased T cell number in the bone marrow is necessary for the increase in osteoclast and the bone wasting induced by ovariectomy (42, 43). Both GHR and IGFR1 have been demonstrated on human peripheral blood mononuclear cells (44–46) and upon activation may directly stimulate the production of TNF- α , IL-6 and IL-1 β from monocytes and macrophages (47, 48). Furthermore, IGF-I stimulates the proliferation of human T cells and activation of IGF-1R by CD28 protects activated T cells from apoptosis (49, 50). Finally, enhanced T cell activity has been found when investigating lymphocyte subsets in acromegalic patients (51).

GH/IGF-I and the OPG/RANK/RANKL axis

Critical for the bone resorptive process is the balance between the newly discovered members of the TNF ligand and receptor superfamilies, osteoprotegerin (OPG) and receptor activator of nuclear factor- κ B ligand (RANKL), which mediate the effects of many upstream regulators of bone metabolism (Fig. 1) (reviewed in 52). *In vitro* experiments show that RANKL stimulates osteoclast differentiation, activates mature osteoclasts, and inhibits osteoclast apoptosis (53–55). In fact, in the presence of macrophage colony stimulating factor (M-CSF) binding to its receptor (c-fms), the ligand is both sufficient and necessary

Figure 1 Osteoblastic cells express soluble and membrane-bound RANKL as well as the decoy receptor for RANKL, OPG, upon stimulation with GH/IGF-I (1). Multipotent cells of the macrophagic lineage proliferate into preosteoclasts and differentiate to become pre-fusion osteoclasts (2) when stimulated with M-CSF and RANKL. OPG blocks the effects of RANKL by neutralizing and preventing binding to its receptor RANK. RANKL stimulates fusion into multinucleated osteoclasts (3) and activates them to resorb bone (4). OPG blocks these effects. OPG induces and RANKL prevents apoptosis of mature osteoclasts (5). RANKL-activated osteoclasts may release IGFs stored in bone matrix during bone resorption (6) and these IGFs may promote a population and activity of osteoblasts (7) in proportion to the volume of bone tissue resorbed (8). In addition, GH/IGF-I may directly stimulate osteoclastic function and activity (9).

for osteoclast formation, and thus resorption. The final piece in the system is the receptor for RANKL, which previously was identified on immune cells and named RANK (56). RANK is present on osteoclasts and their precursors and when bound activates several signaling pathways leading to biological activity. OPG blocks the effects of RANKL by neutralizing and preventing binding to its receptor RANK.

Insulin receptor substrates (IRS-1 and IRS-2) are essential for intracellular signaling by insulin and IGF-I, and therefore the anabolic effects of these on osteoblasts. Mice lacking the IRS-1 or IRS-2 gene both exhibit osteopenia by different mechanisms involving osteoblastic regulation of RANKL (57, 58). Whereas IRS-2 $-/-$ mice are characterized by an uncoupling state with decreased bone formation and increased bone resorption, partly due to increased osteoblastic production of RANKL (57), IRS-1 $-/-$ mice display a low-turnover osteopenia characterized by decreased bone formation and RANKL-induced bone resorption (58). Hence, IRS-1 and IRS-2 seem to have opposite effects on osteoclastogenesis and RANKL expression in osteoblasts and a concerted regulation of these mediators by IGF-I plays a key role in controlling bone metabolism and maintaining bone homeostasis.

Increased OPG in serum from postmenopausal women was recently reported, suggesting that OPG may be regulated by age-related factors such as GH/IGF-I (59, 60). Moreover, a similar age-related increase in the bone matrix accumulation of OPG in postmenopausal women has been demonstrated, significantly negatively correlated with bone mass in cortical bone, indicating that although OPG is produced in many tissues, bone-derived OPG may be released into the circulation, and serum levels may be a parameter of enhanced activity and turnover in the OPG/RANKL/

RANKL system (61). The significance of bone matrix OPG is largely unknown. The increase in serum OPG found in metabolic bone disease may be compensatory to increased osteoclastic bone resorption. Thus, OPG may be released from storage during excess resorption or released from osteoblasts directly to attenuate osteoclastic activity and compensate for increased bone resorption by binding RANKL and blocking RANK activation. Still, OPG does not seem to be a marker of bone turnover since serum OPG levels were normal in patients with acromegaly, as well as GHD (62). Furthermore, no changes in serum OPG were seen during GH substitution in aoGHD women (29) or elderly (63). Another study found increased serum OPG following GH substitution in a mixed population of patients with GHD, negatively correlated to changes in bone turnover (64). *In vitro*, Rubin *et al.* (63) found that IGF-I increased RANKL and decreased OPG expression in mouse stromal cells, favoring pro-resorptive activity *in vitro*. Furthermore, they found that IGF-I treatment to postmenopausal women decreased serum OPG. In contrast, using the same cell line, Gorny *et al.* (65) found that IGF-I increased OPG expression although the RANKL/OPG ratio increased. These differences observed during *in vivo* treatment with GH and IGF may have several explanations. First, serum IGF-I was higher in subjects treated with IGF-I compared with GH (63). Secondly, there may be alterations in free IGF-I in the different studies, as GH markedly increases IGFBP-3 (66). Thirdly, the response to GH or IGF-I may differ depending on the population studied (postmenopausal women or GHD). Finally, based on the known effects estrogen has on OPG protein and gene expression *in vivo* and *in vitro* (67, 68), the effects of estrogen replacement therapy may mask effects of GH/IGF on circulating OPG and explain the unchanged levels in some treatment studies.

Still, serum levels may not necessarily reflect the cytokine levels in the bone microenvironment and *in vitro* models may not account for other OPG-regulating cytokines influenced by GH/IGF-I. Thus, increased OPG protein and gene expression has been demonstrated in cortical bone explants following GH substitution, reflecting the *in vivo* situation locally in bone (28, 29). Nonetheless, increased cortical OPG expression may protect against GH/IGF-I-induced bone resorption and potentially be important for the long-term beneficial effects of GH replacement. Calcitropic hormones, cytokines and growth factors often regulate the expression of both OPG and RANKL from osteoblasts and therefore the ratio of these may ultimately determine the effect of GH/IGF-I on osteoblast-mediated bone resorption. However, this ratio may be difficult to interpret, at least *in vivo*, for several reasons. Although differences in this ratio may exist in serum from different patient groups, OPG is present in excess on a molar ratio (as is typical for TNF ligands and receptors) making results difficult to interpret. Also, the role of soluble RANKL is unknown as it is the membrane-bound species that is the most potent in regulating osteoclastogenesis. Finally, the influence of TNF-related apoptosis-inducing ligand, a cytotoxic protein that may bind OPG (69), on this equation is unknown. Further studies investigating the OPG/RANKL ratio and system in transgenic GH/IGF models may clarify these issues.

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