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Effects of Growth Hormone and Its Secretagogues on Bone

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The growth hormone (GH)/insulin-like growth factor-1 axis is not only of importance for linear body growth during childhood, but it is also one of the major determinants of adult bone mass. Studies show that GH treatment increases bone mass in rodents as well as in adult GH-deficient humans, but the effect of GH treatment on bone mass in healthy humans has so far not been impressive. Recently, a new class of GH secretagogues (GHSs) has been developed. In humans, GHS treatment affects biochemical markers of bone turnover and increases growth velocity in selected short children with or without GH deficiency. In rodents, GHS treatment increase bone mineral content, but it has not yet been shown that GHS treatment can affect bone mass in adult humans.

Key Words: Growth hormone; growth hormone secretagogue; bone; bone mineral content; bone mineral density.

Effects of GH on Longitudinal Bone Growth

(GH) Growth hormone is of major importance for linear bone growth. GH transgenic mice may grow to double the size of their normal littermates *(1)*. Since the late 1950s, it has been known that GH treatment increases linear bone growth in GH-deficient children *(2)*. GH acts directly on bone, as shown in a study by Isaksson et al. *(3)*, in which local injections of GH into the rat tibia growth plate stimulated longitudinal bone growth at the site of injection. Subsequent studies have shown that GH stimulates prechondrocytes in the rat growth plate *(4, 5)*. Furthermore, GH increases local insulin-like growth factor-1 (IGF-1) mRNA expression in bone *(6)*.

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Effect of GH on Bone Formation and Bone Resorption

GH is of importance for bone remodeling *(7)*. Bone remodeling is a coupled process of bone formation and bone resorption that occurs continuously throughout the skeleton in microscopic remodelling units. In the remodeling units, old bone is removed by osteoclasts and new bone is formed by osteoblasts. In acromegaly, biochemical markers of both bone formation and bone resorption are increased *(7)*. In GH-deficient adults, biochemical markers of bone turnover have demonstrated both normal *(8)* and decreased *(9)* rates of bone remodeling. The scarce histomorphometry data on GH-deficient adults have shown an increased eroded surface, osteoid thickening, and increased mineralization lag time, suggesting a prolonged reversal phase, delayed coupling, or a delay in the mineralization process *(10)*. Therefore, adult GH deficiency is most probably a state of low bone turnover. GH treatment has consistently increased biochemical markers of both bone formation and bone resorption in GH-deficient adults *(11,12)*, as well as in non-GH-deficient adults *(13,14)*.

The mechanisms for the effect of GH on bone remodeling are not fully understood. However, rodent as well as human osteoblasts express functional GH receptors, suggesting that GH can stimulate bone formation via a direct stimulation of osteoblasts *(15,16)*. Furthermore, at least some of the effect of GH on osteoblasts may be mediated through local stimulation of IGF production because IGFs are expressed in osteoblasts and exert anabolic effects on these cells *(7)*. The bioactivity of the IGFs in bone tissue is modulated by several IGF-binding proteins (IGFBPs), mainly IGFBP-3, IGFBP-4, and IGFBP-5 *(7)*. GH increases IGFBP-3 production and IGFBP-5 mRNA in rat osteoblasts *(17,18)* whereas no effect of GH is seen on IGFBP-3 expression in human osteoblasts *(15,19,20)*. GH treatment increases serum levels of IGFBP-3 and IGFBP-5 *(21)*.

GH may also regulate bone remodeling by modulating bone resorption. An effect of GH on bone resorption is supported by the fact that GH increases the number of osteoclasts in the metaphyseal bone of the proximal tibia of hypophysectomized rats *(22)*. In a study by Nishiyama et

al, using mouse stromal cells and hemopoietic blast cells, GH stimulated osteoclastic bone resorption through both direct and indirect actions on osteoclast differentiation and indirect activation of mature osteoclasts *(23)*. Furthermore, GH increases interleukin-6 (IL-6) mRNA levels as well as IL-6 protein released to the culture medium from human osteoblast-like cells *(24)*. This could suggest that GH indirectly, via a regulation of IL-6 production in osteoblasts, may regulate bone resorption.

Effects of GH on Bone Mass in Animals

In young rats, GH treatment increased bone mass by increased longitudinal growth at the growth plate and by increased subperiosteal bone formation *(7)*. In old male rats, GH treatment for 80 days increased cortical bone formation mainly owing to increased subperiosteal bone formation *(25)*. In both young and old rats, GH treatment increased cortical bone mechanical strength, mainly due to an increase in bone dimensions *(25,26)*. Eighty-four days of GH treatment in adult dogs increased skeletal mass *(27)*. In primates, GH but not IGF-1 given to female monkeys for 7 wk increased bone formation as measured with mineral apposition rate and bone formation rate *(28)*.

GH treatment increased bone mineral content (BMC) in rodents whereas the volumetric bone mineral density (BMD) (BMC/volume) was not increased *(25,26,29)* as measured by Archimedes' principle or peripheral quantitative computed tomography. By contrast, using dual X-ray absorptiometry (DXA), an increased area BMD (BMC/area) was reported after the administration of GH *(30)*. It is possible that DXA overestimates the increase in BMD during GH treatment since it does not account for the increase in bone mass perpendicular to the DXA image. Therefore, when cortical bone mass is increased because of enhanced subperiosteal bone deposition by GH, this may in itself result in an increased area BMD as measured by DXA.

In all these experiments, the GH-treated rats gained weight. However, it is unlikely that an increased mechanical load owing to increased weight explains the increase in bone mass during GH treatment. When GH was administered during weightless conditions (space flight), the increase in subperiosteal bone deposition was similar to that obtained when GH was administered during weight conditions (on the ground) *(31)*.

Effects of GH on Bone Mass in Humans

In acromegaly, most studies suggest that cortical bone mass is increased whereas cancellous bone is unaffected *(32–34)*. Adults with childhood-onset GH deficiency have reduced BMC and BMD *(35,36)*. Low bone mass is also found in adulthood GH deficiency *(37,38)*; however, one study in elderly GH hypopituitary subjects over the age 60 found a normal BMD compared with healthy volunteers *(39)*. During short-term (<12 mo) GH treatment of GH-deficient adults, bone mass was unchanged or even decreased *(40–42)*. However, long-term *(12–18* mo*)* GH treatment increased BMD in adult GH deficiency of both childhood and adult onset *(36,42–44)*. However, as found in the study by Johannsson et al. *(44)*, the effect on BMC is more marked than the effect on BMD. In healthy adults, the effects on bone mass by GH has not been impressive.

Effects of GH on Incidence of Fracture

Two studies now indicate increased risk of fractures in hypopituitary adults with untreated GH deficiency. Wüster et al. *(45)* found an increased prevalence of vertebral fractures among hypopituitary adults compared with normal controls assessed by using X-ray. Using questionnaires, Rosén et al. *(46)* found an increased prevalence of fractures in 107 hypopituitary adults compared with a reference population.

GHS Treatment

As already discussed, there is still no evidence that GH treatment can increase bone mass in adult humans without GH deficiency. However, the potent effects by GH in experimental animals as well as in GH deficiency suggest that long-term GH may exert a stimulatory effect on bone mass in humans. However, the use of GH is much limited because GH must be administered as sc injections. Therefore, it may be more appropriate to increase circulating GH concentrations by administering an orally active GH secretagogue (GHS). GHSs mimic a recently discovered endogenous hormone, ghrelin *(47)*. They act on a specific receptor *(48)*, thereby stimulating GH-releasing hormone (GHRH) release from the hypothalamus into portal blood *(49)*. GHSs may also act as functional somatostatin antagonists at the pituitary level *(50)*. By these actions, GHSs stimulate the physiologic, pulsatile GH secretion *(50)*. Some of the GHSs are capable of GH release also after oral administration *(50)*.

There is now evidence that GHS treatment can exert anabolic effects. Treatment with the GHS MK-677 increased muscle strength during remobilization after hindlimb immobilization in beagles *(51)*. In healthy volunteers, MK-677 treatment reversed diet-induced nitrogen loss *(52)*. In addition, in healthy obese subjects, GHS treatment induces a substantial increase in fat-free mass *(53)*.

In old beagles, hexarelin treatment reduced the urinary concentration of lysylpyridinoline, a marker of bone resorption *(54)*. Hexarelin treatment counteracted bone loss in gonadectomized male rats *(55)*. Of interest, and unlike GH, hexarelin treatment inhibited markers of bone resorption (55). Ipamorelin treatment has been shown to induce longitudinal bone growth in rats *(56)*. In another study, ipamorelin and GH-releasing peptide-6 treatment increased cortical and total bone mass to an extent similar to GH itself *(57)*. Similarly, as found during previous studies in rodents,

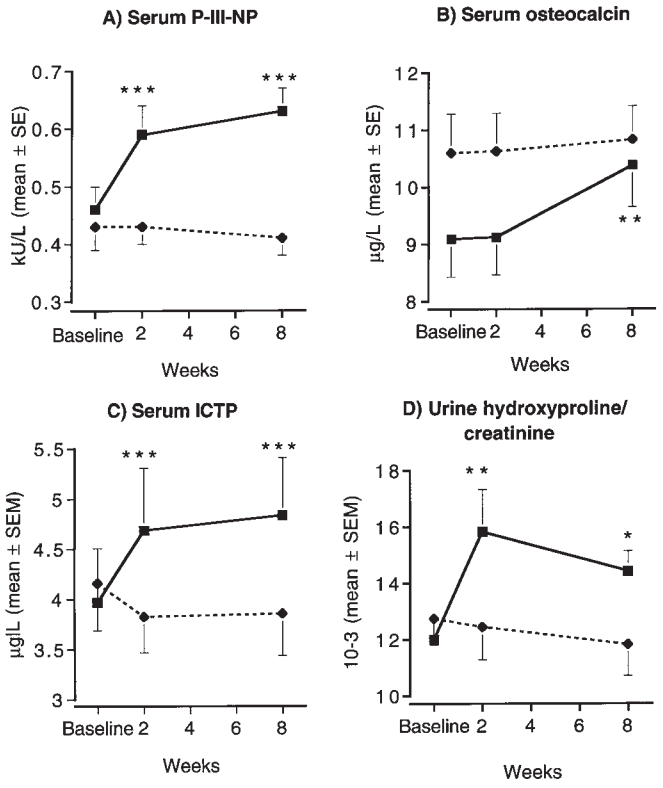

Fig. 1. (**A**) Serum procollagen-III-peptide (P-III-NP); (**B**) serum osteocalcin; (**C**) serum type I collagen telopeptide (ICTP); and (**D**) urine hydroxyproline/creatinine ratio during 2 mo of daily treatment with 25 mg of MK-677 — ■ — or placebo – – ◆ – – in obese men. * $p < 0.05$; ** $p < 0.01$; *** $p \leq 0.001$.

the secretagogues as well as GH increased the bone dimensions whereas the volumetric BMD was unchanged *(57)*.

In humans, several studies show that GHSs are capable of increasing growth velocity in selected children of short stature with or without GH deficiency *(58–60)*. In healthy elderly subjects, hexarelin treatment did not affect body composition or markers of bone resorption where the serum level of the C-terminal propeptide of collagen I (a marker of bone formation) was increased *(61)*. In healthy obese subjects *(62)* as well as in healthy and functionally impaired elderly subjects *(63)*, MK-677 treatment induced potent increases in biochemical markers of bone formation and bone resorption. In the 2-month study in healthy obese subjects *(62)*, MK-677 treatment elicited a rapid induction of markers of bone resorption whereas the increase in markers of bone formation were more marked at study end (**Fig. 1**). This is in line with the previously described biphasic model of GH action in bone *(7)*. According to that model, GH initially increases bone resorption with an increased number of bone-remodeling units and an increased amount of newly produced unmineralized bone. After an initial loss of bone, bone formation is stimulated more than bone resorption and a net gain of bone mass will be found (in GH- deficient adults after 12–18 mo of GH treatment) *(7)*.

Conclusion

GH treatment has consistently increased markers of bone formation and bone resorption. In rodents as well as in GH-deficient adults, GH treatment increases BMC and, to a lesser degree, BMD as measured by DXA, but it is not yet convincingly shown that GH increases bone mass in healthy humans. GHS treatment has been shown to increase BMC in rodents and growth velocity in selected short children with or without GH deficiency. Nonpeptidyl GHS (MK-677) increases markers of both bone formation and bone resorption, whereas several studies suggest that peptidyl GHS (hexarelin) does not affect or even inhibit bone resorption.

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