GH replacement therapy increases plasma osteoprotegerin levels in GH-deficient adults

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Abstract

Objective: Osteoprotegerin (OPG), a glycoprotein belonging to the tumor necrosis factor receptor family, is an endogenous inhibitor of osteoclastogenesis produced by cells of the osteoblast lineage. OPG is a key cytokine involved in the regulation of osteoblast/osteoclast cross-talk. Since GH replacement therapy in GH deficiency (GHD) activates bone remodeling and increases bone mass, we investigated if short-term GH replacement therapy affects plasma OPG levels.

Design and methods: Eighteen adults with GHD, ranging from 17 to 51 years (nine childhood-onset and nine adult-onset) were enrolled in the study. All subjects were on stable replacement therapy, especially sex hormones. The starting dose of GH replacement therapy was 4 mg/kg per day £ 7 days/week, and was progressively increased according to the serum IGF-I values. Biochemical parameters of bone and mineral metabolism were measured before and after 6 months of GH replacement therapy. Bone mass density (BMD) was monitored at three skeletal sites (lumbar vertebrae, femur, radius) by dual-energy X-ray absorptiometry.

Results: After 6 months of therapy, ionized calcium, parathyroid hormone and 25-OH vitamin D did not change, whereas total serum calcium and urinary calcium excretion increased significantly (P < 0.01). Also osteocalcin and urinary deoxypyridinoline/24 h increased significantly (P < 0.02, P < 0.05 respectively). Mean basal T-scores of BMD values showed an osteopenic state, which remained unchanged after GH therapy. Plasma OPG increased significantly after 6 months of therapy (P < 0.02) and this increase was significantly correlated with the increase of osteocalcin (r = 0.52; P = 0.04) and deoxypyridinoline values (r = −0.64; P = 0.011).

Conclusions: Our results suggest that the bone anabolic effect of GH replacement therapy could in part be mediated by a positive bone balance at each remodeling unit due to the inhibitory action of OPG on osteoclastogenesis.

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Introduction

Bone remodeling is characterized by a sequence of hierarchical cellular events devoted to the substitution and maintenance of the bone mass units in the adult skeleton. This process is regulated by a concert of local and systemic factors among which growth hormone (GH) plays a pivotal role by exerting a stimulatory action on both osteoblasts and osteoclasts. When GH-deficient (GHD), adults develop an osteoporotic/osteopenic state characterized by a low rate of bone turnover (1–8) and a 3-fold increase in fracture rate (9). On the other hand, GH replacement significantly increases bone turnover, and induces a biphasic pattern of bone mass changes (10) with early absent or even adverse effects on bone mass density (BMD) within the first 6–12 months (11–14) followed by anabolic ones later on (15–19). It is therefore likely that GH effects in adults with GHD are derived from a complex interaction between GH-dependent factors (insulin-like growth factors (IGFs), IGF-binding proteins etc.) and the level of the cross-talk between osteoblasts and osteoclasts, which regulates the amount of bone formation following each cycle of bone resorption. The recent identification of the osteoprotegerin (OPG)/receptor activator of nuclear factor kappa B (NF-κB) ligand (RANKL)/receptor activator of NF-κB (RANK) system (20) as the dominant mediator of osteoclastogenesis under osteoblast control could throw light on the cellular events underlying the different effects on bone of GH replacement in GHD adults. OPG is a tumor necrosis factor (TNF) receptor superfamily
member, produced by osteoblastic lineage cells in a monomeric (55 kDa) or dimeric (110 kDa) form. Lack- ing transmembrane and cytoplasmic domains, OPG is secreted as a soluble protein (21, 22) and acts as a decoy receptor for RANKL, an osteoblastic lineage cell-derived promoter of osteoclast differentiation and activation (23). OPG acts as a competitive inhibitor of the interaction between RANKL and its osteoclast surface receptor RANK (24). OPG production is controlled by a number of well-known factors regulating bone mass: interleukin-1 and -18 and transforming growth factor-β1 up-regulate OPG mRNA expression (25–27), whereas TNF-α and estrogen increase both OPG expression and secretion (28–30). In contrast, parathyroid hormone (PTH) (31), prostaglandin E2 (32), and the immunosuppressant cyclosporin A (33) inhibit the expression of OPG mRNA, while glucocorticoids also reduce OPG secretion (34). Since preosteoblast/stromal cells display specific receptors for the GH/IGF axis, it is conceivable that the restoration of the GH/IGF axis could stimulate OPG production by these cells and subsequently modulate bone formation/bone resorption coupling. In fact, recent observations show that OPG levels in trabecular and cortical explants from GHD patients are enhanced by GH therapy (35). However, it is still unknown if this enhanced OPG level could be detected by plasma measurements. In fact it has been observed that patients with GHD or GH excess (acromegaly) do not differ significantly in their circulating OPG levels (36).

Therefore, we designed the following open longitudinal study with the aim of evaluating circulating OPG levels under GH replacement therapy and to analyze their relationship with bone remodeling markers. For these purposes, GHD adults underwent a complete assessment of their bone and mineral metabolism before and after a short course of GH therapy.

Subjects and methods

Subjects and experimental procedures

Eighteen consecutive patients with adult GHD referred to the Departments of Endocrinology and Neurosurgery at San Raffaele Hospital participated in the study. Patients characteristics are shown in Table 1. Diagnosis of adult GHD was based upon a maximum peak GH response of less than 9 μg/l to stimulation by arginine (0.5 g/kg i.v.) and GH-releasing hormone (50 μg i.v.) (37) assessed in patients with a history of previous pituitary surgery or radiotherapy or presenting a history of childhood-onset GHD. Patients were receiving adequate and stable replacement therapy with gonadal, thyroid and/or glucocorticoid hormones, when needed, before starting GH therapy. The GH starting dose was 4 μg/kg per day s.c., progressively increased accordingly to the serum IGF-I values. At the start of GH therapy three patients were already receiving vitamin D/calcium supplementation.

Blood samples for IGF-I, total and ionized calcium, phosphate, PTH, 25-OH vitamin D (25(OH)D), osteocalcin (BGP) and OPG were drawn in basal conditions and 6 months after the start of GH therapy. Twenty-four hour urine collections for deoxypyridinoline (Dpd), calcium and phosphate excretion determination were taken at the same times. The renal tubular reabsorption of phosphate was calculated using the Walton and Bijvoet nomogram and expressed as

Table 1 Clinical characteristics of the subjects enrolled in the study.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Sex (M/F)</th>
<th>Age (years)</th>
<th>Age of GHD onset</th>
<th>Pathology</th>
<th>ACTH</th>
<th>LH/FSH</th>
<th>TSH</th>
<th>ADH</th>
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<tr>
<td>1</td>
<td>M</td>
<td>27</td>
<td>Childhood</td>
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<tr>
<td>2</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>19</td>
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<td>–</td>
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<td>4</td>
<td>F</td>
<td>16</td>
<td>Childhood</td>
<td>Congenital defect</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<td>5</td>
<td>M</td>
<td>16</td>
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<td>–</td>
<td>+</td>
<td>–</td>
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<td>6</td>
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<td>+</td>
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<td>+</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
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<tr>
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<tr>
<td>13</td>
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<tr>
<td>14</td>
<td>M</td>
<td>46</td>
<td>Adulthood</td>
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<tr>
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</tr>
<tr>
<td>17</td>
<td>M</td>
<td>28</td>
<td>Adulthood</td>
<td>Pituitary macroadenoma</td>
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<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>23</td>
<td>Childhood</td>
<td>Craniohypophygioma</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

ACTH, adrenocorticotropic; LH, luteinizing hormone; FSH, follicle-stimulating hormone; TSH, thyrotropin; ADH, antidiuretic hormone.
the ratio of renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR) (38).

BMD was assessed in basal conditions, and at 6 months of GH therapy.

The study protocol was approved by the local Institutional Ethical Committee.

**Assays**

Serum IGF-I levels were measured with a RIA (IGF-I Kit; Bioclone, Australia). The sensitivity of the measurement was 0.018 μg/ml; intra-assay coefficients of variations (CVs) were 2.9% (at lower range) and 5.4% (at upper range); the interassay CVs were 4.3 and 7.1%, respectively. Total serum and urinary calcium, phosphate and creatinine were measured colorimetrically (Roche Diagnostics, Monza, Italy), using an Hitachi 917 autoanalyzer (Hitachi, Tokyo, Japan). Serum ionized calcium was measured by means of a selective electrode (AVL; Roche Diagnostics), its intra- and interassay CVs being 2 and 3%, respectively. Plasma intact PTH levels were measured with an IRMA (Incstar Corp., Stillwater, MN, USA); both CVs, within the assay and among assays, for PTH values within the reference range were less than 6%. Plasma 25(OH)D levels were measured with a RIA after extraction (Diasorin, Saluzzo, Italy); intra- and inter-assay CVs were 4 and 5%, respectively. Serum BGP levels were determined with an IRMA (Nichols Institute, San Juan De Capistrano, CA, USA); the intra- and interassay CVs were 5.1 – 7.1 and 3.2 – 5.2%, respectively. Plasma OPG levels were assessed by sandwich-type ELISA (Immunodiagnostik AG, Bensheim, Germany); inter- and intra-assay CVs were lower than 10% and the detection limit was 0.14 pmol/l.

Total urinary Dpd was measured by ion-pair reverse-phase HPLC after hydrolysis of the diluted urines and extraction with partition chromatography (BioRad Laboratories, Segrate, Italy). The analyte was detected fluorimetrically (excitation 295 nm; emission 400 nm) utilizing its natural fluorescence. The within-run CV was 5%.

**BMD**

BMD was assessed by dual-energy X-ray absorptiometry (Hologic QDR 4500 W) at L1–L4 lumbar spine vertebrae, femur and radius. The BMD values were expressed as the number of S.D. from the mean of a healthy young Caucasian population (T-score). Patients having T-scores equal to or lower than –2.5 were defined as osteoporotic; patients with T-scores between –2.5 and –1 were defined as osteopenic; patients with T-scores equal to or higher than –1 were defined as normal. The CVs for the instrument were calculated daily by quality control scans of spine phantom and were lower than 0.5%.

**Statistics**

All results are presented as means±S.E.M. All statistical comparisons were performed by paired Student’s t-test, with the exception of 25(OH)D and urinary Dpd, which were compared by the Wilcoxon signed-rank test, due to the non-normal distribution of the data, assessed by the Kolmogorov–Smirnov test. Correlation between increments was calculated by means of the Spearman rank correlation test. P < 0.05 was considered statistically significant.

**Results**

After 6 months of GH replacement therapy, serum IGF-I levels were significantly enhanced (P < 0.0001) as compared with basal levels and returned to within normal range for sex and age in all patients (Fig. 1). Basal evaluation of bone and mineral parameters, besides osteopenia, did not reveal any major metabolic disturbances, with the exception of low mean 25(OH)D values, which were below the accepted threshold for vitamin D sufficiency (15 ng/ml) (39), and of four cases of secondary hyperparathyroidism.

At 6 months of GH therapy, no significant changes in the plasma 25(OH)D levels were detected. Mean plasma PTH levels, as well as the ionized Ca/PTH relationship, did not change significantly; the high PTH basal values returned to within the normal range after 6 months of GH therapy while increasing in two other different subjects (Fig. 2). Total calcium levels were significantly higher (P < 0.02) as compared with basal levels, whereas ionized calcium levels remained unchanged. The 24 h calcium excretion and the TmP/GFR, were significantly increased after treatment (Table 2). BGP levels and 24 h Dpd excretion increased significantly (P < 0.02, P < 0.05 respectively) with respect to basal values (Fig. 3).

Plasma OPG values significantly (P < 0.02) increased after 6 months of GH therapy (Fig. 4).
The OPG increment ($\Delta$OPG = OPG$_{6 \text{ month}}$ − OPG$_{\text{basal}}$) did not correlate with the enhancement of the IGF-I concentration whereas it did negatively correlate with the increase observed for BGP ($\Delta$BGP = BGP$_{6 \text{ month}}$ − BGP$_{\text{basal}}$) ($r = -0.52; P = 0.04$) and for Dpd excretion ($\Delta$Dpd = Dpd$_{6 \text{ month}}$ − Dpd$_{\text{basal}}$) ($r = -0.64; P = 0.011$) (Fig. 5).

BMD at lumbar spine, femoral and radial sites remained unmodified (Table 2).

**Discussion**

This study has demonstrated that short-term GH replacement therapy in GHD adults is associated with a significant increase in the plasma OPG levels. It is conceivable that the OPG increment is due to the GH action, and not to the concomitant hormonal replacement therapies, or to the effect of any change of the mineral metabolism parameters because: (i) before the basal assessment of plasma OPG all patients were under continuous and adequate hormone replacement therapy in order to be in endocrine equilibrium; and (ii) mean PTH concentration was unmodified under therapy as well as the ionized Ca/PTH relationship. Since the sporadic variations in PTH concentration observed in some patients during therapy were in opposite directions, it is highly unlikely that they could explain the consistency of the OPG increments (−90% of the patients).

This study has confirmed the stimulatory action of GH replacement upon the activation frequency of bone remodeling (40, 41) that did in fact take place at 6 months of therapy. The concomitant increase of both BGP and Dpd rule out a selective stimulation of bone formation as a potential factor responsible for the late anabolic effect of GH therapy on bone mass of adults with GHD (15−19).

The enhancement of plasma OPG levels under GH therapy probably reflects an enhanced OPG production at the bone level in GHD patients. This view is.

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**Table 2** Additional parameters before and after 6 months of GH therapy.

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Six months</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>102.94+$\pm$16.93</td>
<td>226.17+$\pm$17.10</td>
<td>$&lt;0.000$</td>
</tr>
<tr>
<td>BGP (ng/ml)</td>
<td>34.8+$\pm$4.5</td>
<td>46.9+$\pm$5.1</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>Ionized calcium (mmol/l)</td>
<td>1.21+$\pm$0.01</td>
<td>1.23+$\pm$0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total calcium (mmol/l)</td>
<td>2.42+$\pm$0.03</td>
<td>2.48+$\pm$0.02</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>49+$\pm$6</td>
<td>44+$\pm$5</td>
<td>n.s.</td>
</tr>
<tr>
<td>25(OH)D (ng/ml)</td>
<td>14.0+$\pm$1.6</td>
<td>13.6+$\pm$2.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mmol/24 h)</td>
<td>4.07+$\pm$0.51</td>
<td>4.86+$\pm$0.64</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Dpd (nmol/24 h)</td>
<td>123.3+$\pm$15.90</td>
<td>150.9+$\pm$20.87</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>TmP (mg/dl)</td>
<td>3.31+$\pm$0.25</td>
<td>4.06+$\pm$0.16</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>T-score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar vertebrae L$_1$–L$_4$</td>
<td>$-1.08+$0.18</td>
<td>$-1.05+$0.18</td>
<td>n.s.</td>
</tr>
<tr>
<td>Femur</td>
<td>$-0.89+$0.32</td>
<td>$-0.68+$0.28</td>
<td>n.s.</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>$-0.90+$0.37</td>
<td>$-0.81+$0.34</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ultradistal radius</td>
<td>$-1.56+$0.34</td>
<td>$-1.60+$0.31</td>
<td>n.s.</td>
</tr>
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</table>
supported by the negative correlation between OPG increment and the increase of both biochemical markers of bone remodeling. It also fits with the recent finding that GH replacement therapy in GHD adults induces OPG accumulation in trabecular and cortical bone explants (35). However, the determination of plasma OPG levels does not seem to have a discriminatory value among populations since OPG levels were found to be not significantly different in acromegalic and GHD patients from healthy controls (36); it could only indicate that the level of cross-talk between bone cells and the OPG/RANKL equilibrium can be regulated by the GH/IGF system in GHD patients as well as in elderly postmenopausal women (42), although the direction of the changes may differ depending upon local and systemic factors acting on the bone remodeling sequence.

This study suggests that OPG is the paracrine mechanism of inhibition of osteoclast formation during GH-enhanced remodeling that might play the key role in determining the positive bone mass outcome of GH therapy. It is in fact likely that, despite the overall activation of bone remodeling under GH stimulus, the relative osteoclast component is reduced by the inhibitory action of OPG on the osteoclastogenesis as indicated by the negative correlation between OPG and Dpd increments. This view is further supported by the negative correlation between OPG and BGP increases, since bone resorption and formation are coupled and BGP serum levels at any one time have a component of both bone resorption and formation. The GH-dependent positive shift of bone balance at each remodeling unit determines increased bone mass and improved mechanical competence.

The inhibitory action of OPG on osteoclastogenesis could represent an homeostatic mechanism activated by GH replacement therapy serving to limit bone resorption. This mechanism, which becomes operative in elderly men as well as in postmenopausal women (43, 44) with enhanced bone remodeling, is regulated by a concert of regulatory factors, besides GH, that participate in bone mass homeostasis. Estrogens are under current debate and might explain the difference in gender sensitivity of GH therapy (45) because of their strong action on OPG production (29, 30). Along this line of thought, it is conceivable that monitoring plasma OPG levels during GH therapy might predict future bone mass changes and help to optimize the effects of the hormonal cofactors to achieve clinical benefits on skeletal structure and integrity.

The secondary hyperparathyroidism that was observed in basal condition was an unexpected finding, not necessarily related to the vitamin D insufficiency. Nevertheless, it normalized or tended to normalize by the effects of both vitamin D supplementation (two cases) and GH-induced positive shift of calcium balance.
as suggested by the significant increase in the 24 h calcium excretion. It is in fact generally acknowledged that GH enhances the availability of the mineral substrates to support bone growth requirements, possibly by acting on calcitriol synthesis (46). It is even likely that GH has a direct regulatory effect on tubular reabsorption of phosphate as suggested by the significant increase in TmP/GFR after therapy, thus confirming previous observations (47).

The secondary hyperparathyroidism that developed in two patients during therapy was due to a negative calcium balance as a possible consequence of enhanced calcium bone clearance not counteracted by adequate oral calcium supplementation and/or absorption. It follows that adequate calcium—vitamin D supplementation might be associated with potential benefits in GHD adults under GH replacement therapy by avoiding the negative effects of secondary hyperparathyroidism on cortical bone mass.

In conclusion, this study showed that OPG plasma levels increased under GH therapy in GHD adults. The enhanced OPG levels suggest that GH therapy resets the increased bone remodeling to a new equilibrium by acting upon the OPG/RANKL/RANK system with subsequent increase of bone mass. These data may be of clinical relevance in monitoring the effects of GH therapy on bone and in optimizing the replacement dose and the endocrine frame to achieve a positive effect on bone mass in GHD adults.

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