Potent effect of recombinant growth hormone on bone mineral density and body composition in adults with panhypopituitarism

Marie Degerblad, Nabil Elgindy\(^1\), Kerstin Hall, Hans-Erik Sjöberg and Marja Thorén

Departments of Endocrinology and Diagnostic Radiology\(^1\), Karolinska Hospital, Stockholm, Sweden


Six patients (21–50 years) with growth hormone deficiency and panhypopituitarism were given recombinant growth hormone, somatropin, 0.04–0.1 U·kg·body wt\(^{-1}\)·day\(^{-1}\), for 12 months. All patients reported improved well-being with increased working capacity. Bone mineral density, as measured by single photon absorptiometry at two sites on the forearm, showed increased values in 5/6 patients after 12 months when measured at the most distal site (predominantly trabecular bone) and in 4/6 at the more proximal site (predominantly cortical bone). Five patients continued therapy for an additional year and after 18 months a significant increase in bone mineral density was seen at both the distal and proximal sites. The mean annual increase in bone mineral density was 12.0 ± 0.6 (SEM)% and 3.8 ± 1.3% at the distal and proximal sites, respectively. In a growth hormone deficient control group without growth hormone therapy, the corresponding values were −2.4 ± 0.6% and −1.9 ± 0.4%, respectively. Lean body mass, estimated anthropometrically, increased significantly after 12 months and total body potassium, measured by whole body counting technique, increased in 4/6 patients. During growth hormone treatment, the IGF-1 values were above the mean values for age and 50% of the values were above the mean ± 2 SD. B-glucose, P-insulin, serum IGF-2, procollagen-III peptide and phosphate increased and urea, creatinine and IGF-binding protein-1 decreased during treatment. The beneficial effects of growth hormone substitution, especially on bone mineral density, indicate that growth hormone substitution should be considered in all patients with hypopituitarism and growth hormone deficiency, irrespective of age.

Marie Degerblad, Department of Endocrinology, Karolinska Hospital, PO Box 60 500, S-104 01 Stockholm, Sweden

Growth hormone therapy in GH-deficient children has been a reality for may years, but such therapy has generally been withdrawn after puberty and achievement of the final height. In healthy subjects, however, GH secretion continues after linear growth has ceased, although the amount of secreted hormone decreases with increasing age (1).

GH has both anabolic and lipolytic actions, and the consequences of many years of GH deficiency during adult life are, thus far, inadequately evaluated. GH-deficient adults have been shown to have increased fat volume and decreased muscle volume (2, 3) as well as decreased lean body mass (4, 5). Bone mineral density has also been shown to be lower than in normalagematched subjects (6, 7). Several factors could influence the low bone mass in these patients. A normal peak bone mass is not reached until several years after puberty and achievement of final height, and this means that the GH-deficient patients are left without GH substitution during a period very important for attaining normal peak bone mass. In patients with several pituitary hormone deficiencies, the difficulties optimizing the induction of puberty and substitution with gonadal steroids, as well as the risk of oversubstitution with cortisone and thyroxine, also make the patients with hypopituitarism extraordinarily disposed to developing osteopenia. The frequency of fractures in older adults with panhypopituitarism is not known. But it appears likely that low bone mineral density may constitute a risk factor for bone fragility and fractures in later life. In addition, adults with panhypopituitarism and adequate thyroxine, cortisone and gonadal steroid substitution therapy, have been shown to have a low quality of life (8) and a decreased life expectancy (9).

The availability of recombinant GH has now made it possible to evaluate the effect of GH substitution therapy with adults having GH deficiency. Recent studies have shown that GH treatment for three to six months in GH-deficient adults improved general well-being (10) and influenced body composition and lean body mass, resulting in increased muscle mass and decreased adipose tissue (2, 3, 5, 11). However, no improvement of the low bone mineral density could be demonstrated after three months of GH treatment (10).

The aim of the present study was to evaluate the effect of GH substitution therapy, over at least one year, on...
bone mineral density and body composition in adults with panhypopituitarism and GH deficiency.

Patients and methods

Patients
Six patients, one man and five women, ranging in age from 21 to 50 (mean 33.0) years, participated in the study (Table 1). All held full-time jobs and were socially well adjusted. Each of the six had panhypopituitarism. Three patients had been treated for craniopharyngiomas, one (no. 6) had received pituitary irradiation 27 years earlier during treatment for Cushing’s disease, and with the two remaining the deficiency was considered idiopathic. All patients received adequate cortisone, thyroxine and sex steroid substitution therapy. Two of the craniopharyngioma patients were also concurrently receiving desmopressin for control of diabetes insipidus. No other drugs were used in any patient during the study period.

Five of the patients (nos. 1–5) had earlier received GH substitution therapy during at least some part of their childhood and adolescence, but none during the last two years. The final height range of the subjects was 153–165 (mean 157 ± 2) cm and their body mass index (BMI) range was 18.4–26.3 (mean 22.0 ± 1.2) kg/m².

The GH deficiency was confirmed by insulin-arginine test and/or a 12 h night GH profile by the continuous withdrawal system. The IGF-1 serum levels of the group ranged from 12 to 151 µg/l and were below mean − 2 SD for age in all subjects. Five patients were considered to have complete and one (no. 1) partial GH deficiency. The latter patient had earlier been considered to be completely GH-deficient according to conventional GH stimulation tests. However, at reevaluation before entry into this study, she had an IGF-1 level of 151 µg/l and was therefore considered as partially GH-deficient.

Study design

The study was of open design. Recombinant GH, somatropin (Genotropin®, kindly supplied by Kabi Pharmacia, Sweden), was administered s.c. by the patient, every evening for 12 months. The initial dose in four of the patients was 0.1 U·kg body·wt⁻¹·day⁻¹. With two of this group (nos. 3 and 4) a reduction in dose to 0.08 U·kg⁻¹·day⁻¹ was made in order to avoid fluid retention. For similar reasons, doses for the remaining two patients (nos. 5 and 6) were established and maintained at 0.06 and 0.04 U·kg⁻¹·day⁻¹, respectively.

The study was approved by the Ethics and Radiations Safety Committees of the Karolinska Hospital and the Swedish Board of Health and Welfare.

Study protocol

Before initiation of the therapy and after 3, 6, 9, and 12 months of GH substitution therapy, routine blood tests and measurements of serum insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-2 (IGF-2) were performed. Serum concentrations of procollagen III peptide, osteocalcin, and insulin-like growth factor binding protein-1 (IGFBP-1) were measured before and after 6 and 12 months GH therapy. At the same time points, measurements of bone mineral density, total body potassium, lean body mass and total body water were carried out.

After 12 months, five of the patients continued with GH therapy and were followed during a second year with routine laboratory tests and measurement of serum levels of IGF-1 as well as measurement of bone mineral density. One patient (no. 1) did not continue GH substitution because of an aversion to injections.

Methods

Bone mineral density. Single photon absorptiometry with radioactive source 125I (Nuclear Data 1100 rectilinear scanning) (11) was used to measure bone mineral density at two sites of the forearm (12). The measurements were started at a point where the distance between ulna and radius is 8 ± 0.8 mm. The first six scans, 4 mm apart, were made proximal to the starting point and measure predominantly cortical bone. The next four scans, 2 mm apart, were made distal to the starting point and measure predominantly trabecular bone. The bone mineral density values were corrected for bone width of radius and ulna at the starting point to compensate for differences in skeletal size (13) and the values were calculated as mean values for right and left arm, proximal and distal measurements, respectively.

Table 1. Patient data.

<table>
<thead>
<tr>
<th>Pat. no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Basal IGF-1 (µg/l)</th>
<th>Etiology of GH deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>f</td>
<td>21</td>
<td>23.1</td>
<td>151</td>
<td>Craniopharyngioma</td>
</tr>
<tr>
<td>2.</td>
<td>m</td>
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<td>18.4</td>
<td>64</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>3.</td>
<td>f</td>
<td>28</td>
<td>23.7</td>
<td>39</td>
<td>Craniopharyngioma</td>
</tr>
<tr>
<td>4.</td>
<td>f</td>
<td>37</td>
<td>20.5</td>
<td>12</td>
<td>Craniopharyngioma</td>
</tr>
<tr>
<td>5.</td>
<td>f</td>
<td>37</td>
<td>26.3</td>
<td>16</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>6.</td>
<td>f</td>
<td>50</td>
<td>19.5</td>
<td>56</td>
<td>Cushing’s disease. Pituitary irradiation</td>
</tr>
</tbody>
</table>

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The precision of the method has been shown to be 1–2% (Naessén T 1991; pers. comm.). The basal bone mineral density values were compared with those of 24 healthy females and 27 males, aged 21–43 (mean 32) years. The basal values in the 50-year-old woman were compared with those in 17 healthy perimenopausal women, 49–53 years old. The basal bone mineral density values are expressed as g/cm² and the values during treatment as the percent of baseline values.

To evaluate changes in bone mineral density over time in patients with panhypopituitarism not receiving GH substitution therapy, bone mineral density was measured in nine patients (seven males), aged 29–42 (mean 36) years, with adequate cortisone, thyroxine and sex steroid substitution therapy. Measurements were repeated after 26–41 (mean 32) months.

Total body potassium, lean body mass and total body water. Total body potassium was determined by the whole body counting technique where the activity of the natural isotope 40K is measured and from which total body potassium can be calculated. The given values are corrected for weight and height in the subjects (14). The predicted values were calculated from measurements in earlier studies of healthy controls (15). Lean body mass was defined as the difference between body weight and total body fat. Total body fat was estimated anthropometrically from the equation

$$TBF = \frac{0.59 \times \text{(weight)}^2 \times \log(\text{skin-fold thickness} - 0.6)}{\text{height}} + 7.1$$

(16). The skin-fold thickness is measured by a caliper and is the sum of the paraumbilical and subscapular skin-folds in centimeters, and 0.6 is the sum of the cutis layers. The error of the method equals 1.8 kg. The formula was derived from measurements of a large group of subjects with normal water and electrolyte metabolism (15). The skin-fold measurements were performed by the same experienced technician at each time point during the study. Total body water was determined by the isotope dilution technique, where 3.7 MBq (100 µCi) of $^{3}H_{2}O$ (Amersham) was given orally (14). After 24 h a blood sample was withdrawn and the plasma radio activity was counted for 20 min in duplicate in an Intertechnique SL 30 liquid scintillation counter. Corrections were made for urinary losses and quenching. The error of the method has been found to be about 3%.

Assays. IGF-1 and IGF-2 were determined by radioimmunoassay (RIA) after dissociation and separation of IGFs from IGF-binding proteins by gel chromatography at low pH (17). Truncated IGF-1 was used as radioligand and recombinant IGF-1 as standard in this IGF-1 RIA utilizing polyclonal rabbit antisera. The mean and range (±2 sd) of IGF-1 serum concentrations in healthy subjects, aged 21–30 years, were 225 and 151–336 µg/l, respectively and in those aged 31–50 years, 162 and 90–294 µg/l, when logarithmic transformed values were used. IGF-2 was determined in a RIA using purified yolk antibodies and recombinant IGF-2 as radioligand and standard. The mean and range (±2 sd) of IGF-2 concentrations in healthy subjects aged 21–70 years were 755 and 539–1058 µg/l, respectively. IGFBP-1 was determined by RIA described by Póvoa et al. (18).

Mean and range in healthy subjects aged 21–50 years were 33 and 12–91 µg/l, respectively, with logarithmic transformed values (19). Procollagen III-peptide in serum was analysed using a commercial RIA kit (CIS International, Gif sur Yvette, Cedex, France) with normal values in adult men <1.9 nmol/l (<11 µg/l), in premenopausal women <1.6 nmol/l (<9 µg/l) and in postmenopausal women <2.4 nmol/l (<14 µg/l).

Statistics

The results are given as mean ± SEM if not stated otherwise. Friedman’s analysis of variance for dependent groups was used when comparing results during GH treatment with basal values. The Mann Whitney U-test was used when comparing bone mineral density results in the patients and the control group. Differences of p < 0.05 were considered to be significant.

Results

All patients reported very positive effects of GH treatment, such as increased alertness, working capacity, muscle strength and the disappearance of hypoglycemic episodes. Fluid retention, which appeared as a swelling of hands and fingers in four subjects and was corrected by a reduction in GH dose in two subjects (the symptom was transient with the other two patients), and transient artralgia in three patients were the only adverse effects reported. Five of the patients requested GH substitution therapy after 12 months. One patient (no. 1) chose to discontinue therapy, mainly because of an aversion to injections.

Biochemical measurements

The mean serum IGF-1 level based on logarithmic values was 40.0 µg/l before GH treatment. After three months of treatment, that mean level was significantly increased to 311 µg/l and remained significantly elevated through the remaining time points of the initial 12-month study period (Fig. 1). With the four patients treated for 24 months, the mean value was 258 µg/l. During GH treatment, the IGF-1 levels were above the mean value for age-matched controls in all individuals and 50% of the IGF-1 values were above mean ± 2 sd.
In Table 2, mean values ± SD for the remaining analyses are shown. Baseline serum IGF-2 levels were below the normal range in four of the six patients (nos. 2-5). The serum levels increased during treatment to values within the normal range in all patients. Fasting blood glucose levels were significantly increased after 12 months of GH therapy when compared to basal values. The concentrations, however, were within the reference range with all patients and the highest individual value observed was 5.1 mmol/l. Fasting plasma insulin concentrations increased significantly showing a twofold elevation above basal levels after 6 and 12 months of GH therapy. The elevated basal IGFBP-1 levels decreased significantly concomitant with the increase in insulin concentration. Serum levels of procollagen III-peptide and phosphate also increased significantly during GH substitution therapy. Serum levels of osteocalcin increased after six months of therapy in four of six patients to values above the reference range and remained elevated after 12 months. In two patients (nos. 1 and 2), the values remained within the normal range. Serum concentrations of urea and creatinine were significantly decreased after 6 and 12 months of treatment when compared to basal levels. No change was observed in the serum levels of albumin, total cholesterol, triglycerides, or serum alkaline phosphatase activity.

**Bone mineral density**

Basal bone mineral density was low in all patients compared to that in healthy subjects. Basal values at the distal and proximal measuring sites in the four younger women were 0.63–0.76 and 1.05–1.15 g/cm² respectively, in the 50-year-old woman 0.76 and 1.24 g/cm² and in the only man 0.92 and 1.38 g/cm². The corresponding values in the healthy controls were: for younger women 0.97 ± 0.01 and 1.33 ± 0.04 g/cm², for the women aged 49–53 years 0.97 ± 0.02 and 1.38 ± 0.04 g/cm² and for the healthy males 1.26 ± 0.03 and 1.60 ± 0.04 g/cm². After six months GH treatment, the mean bone mineral density values were 99.8 ± 1.0 and 98.3 ± 1.5% of the initial values at the distal and proximal measuring sites respectively (Fig. 2). After 12 months, bone mineral density at the most distal site was increased in five patients and unchanged in the sixth (no. 6), when compared to basal values (mean 107.3 ± 2.1%, N = 6). After 18 months, a significant increase to 118.0 ± 6.1% (p < 0.05) was seen.
in the five patients treated for that period. If the oldest patient (no. 6) is excluded, a highly significant increase was observed after 12 months \( (p < 0.001) \) with a further increase after 18 months \( (p < 0.001 \text{ in comparison to basal densities}) \). After 24 months the individual values ranged between 105 and 140% of basal values. At the proximal measuring site, the values were increased after 12 months in four of six patients (mean 103.2 ± 1.5%, \( N = 6 \)). A significant increase was noted at the 18 months time point (mean 105.3 ± 2.0%, \( p < 0.05 \)). If patient no. 6 is excluded, a significant mean increase occurred after 12 months \( (p < 0.01) \) with a further increase observed at 18 months \( (p < 0.001 \text{ when compared to basal densities}) \). After 24 months the values were elevated in all four patients treated for that period (105–114% when compared to basal values). With patient no. 6, the 50-year-old woman treated with the lowest GH dose, the 12 and 18 month values at the distal site were 100 and 99%, respectively, and the corresponding values of the proximal site were 98 and 100%. The mean annual increase in bone mineral density calculated from the 18 months values was 12.0 ± 4.1% and 3.8 ± 1.3% at the distal and proximal measuring sites, respectively. No dose-related effect of GH on bone mineral density could be observed in the patients. The nine “control patients” with panhypopituitarism, who where not receiving GH, exhibited mean annual decreases of 2.4 ± 0.6 and 1.9 ± 0.4% at the distal and proximal measuring sites, respectively, calculated from the values at follow-up. The values at both sites differed significantly between the two groups \( (p < 0.01) \).

Total body potassium, lean body mass and total body water

The basal levels of total body potassium (mean 89.6 ± 7.7 g) were below the predicted value in only two patients. After six months the mean value was 93.8 ± 6.6 g (n.s.). After 12 months the values were increased in five of six patients \( (101.2 \pm 6.8 \text{ g, } N = 6) \). A significant increase in mean lean body mass when compared to basal value was observed after 12 months \( (39.0 \pm 1.5 \text{ and } 42.1 \pm 1.9 \text{ kg, respectively } (p < 0.01)) \). In four of six patients, total body water was markedly increased after 6 and 12 months when compared to baseline \( (41.2 \pm 2.4, 39.0 \pm 2.4 \text{ and } 31.9 \pm 1.5 \text{ l, respectively}) \). Total body water was unchanged with the remaining two subjects. The body weight was not significantly changed during GH substitution therapy.

Discussion

The effect of GH on body composition is well documented and could be verified in this study, too. The most promising effect was the increase in bone mineral density measured by single photon absorptiometry over the distal forearm. A treatment period with GH of at least 12 months seemed necessary before an increase in bone mineral density in the distal forearm could be detected by single photon absorptiometry, where both trabecular and cortical bone are measured at the site. Preliminary reports from v der Veen and co-workers indicate that an improved bone mineral density could be registered in the lumbar vertebrae after six months of GH therapy (6 U daily), when dual photon absorptiometry was used for the measurements of bone mineral density (20). It is possible that a GH induced increase of bone mass in GH-deficient adults is most rapidly induced in trabecular bone and therefore is detected earlier in lumbar vertebrae than in the distal forearm. In this context it must be noted that the GH doses \( (0.28–0.70 \text{ U·kg}^{-1} \text{·week}^{-1}) \) given in the present study induced an increase of IGF-1 to levels above the mean of healthy subjects aged 20–29 years. There was, however, no significant correlation between the serum IGF-1 increase and the individual GH doses given. It may be of value to monitor the GH therapy guided by determinations of serum IGF-1 levels. In parallel with the elevation of IGF-1 levels, a highly significant increase in IGF-2 and procollagen III-peptide concentrations occurred during GH treatment. The elevation of GH-independent IGF-2 is probably secondary to a GH and IGF-1 induced increase of IGFBP-3. A GH dose dependency of procollagen III-peptide has recently been demonstrated by Jensen and co-workers (21).

The GH-induced improvement of the low bone mineral density in patients with hypopituitarism raises the question of whether other forms of osteopenia can benefit from such treatment. While no effect on bone mineral density was observed during six months’ GH treatment given in daily doses of 3.5–5.9 U in patients with postmenopausal osteoporosis (22), the treatment period was probably too short to induce measurable changes of bone mineral density in the distal forearm. Whether patients with postmenopausal osteoporosis have lower GH secretion and IGF-1 levels than the age-matched general population has not been definitely settled.
GH treatment is expected to be most rewarding in those osteoporotic patients who have the lowest GH secretion. In healthy adults, GH secretion declines by age with large interindividual variations. At the age of 65, about half of the population could be considered partially or totally GH-deficient when their GH production is compared with that found in healthy adults during the third decade (1). When 11 healthy elderly, selected for low IGF-1 levels, were treated with GH in a weekly dose of approximately 0.23 U/kg divided into three injections, age-related changes in body composition, such as reduction of muscle volume and increase in adipose tissue, were reversed (23). Their IGF-1 levels reached the normal range for young adults and a small increase (p<0.05) in bone mineral density of lumbar vertebrae was reported after six months’ GH treatment (23).

The GH effect on bone density is most likely to be mediated through IGF-1 acting in both endocrine and paracrine fashion. GH stimulates the expression of IGF-1 by rat osteoblasts and the growth promoting effect of IGF-1 in vitro is blocked by IGF-1 antibodies (24). IGF-2, similar to IGF-1, stimulates proliferation and collagen synthesis of human osteoblasts (25). In addition, IGF-2 is the major skeletal growth factor found in human bone (26), and the concentration of GH independent IGF-2 is more than tenfold higher than that of IGF-1 in rat bone conditioned medium (27). Therefore, it is difficult to attribute the GH effect to only the paracrine action of bone-derived IGF-1. Endocrine IGF-1 may also mediate the GH effect on bone in humans or GH could regulate some bone derived IGF binding proteins, which subsequently modulate the action of paracrine IGF-2.

The patients participating in this study reported improved well-being and improved physical capacity during the treatment period. Apart from initial fluid retention and artralgia in some patients, no adverse effects were encountered. Fasting blood glucose levels increased during treatment, but remained within the normal range in all patients during treatment.

In conclusion, the beneficial effects of GH therapy in adults with panhypopituitarism favour substitution therapy being considered in all ages in these patients. With regard to the substantial improvement of bone mineral density, the effects of GH treatment during other conditions involving osteopenia should be evaluated.

Acknowledgments. The study was supported by grants from the Karolinska Institute, the Swedish Medical Research Council (nos. 4224 and 5992), Svenska Sällskapet för Medicinsk Forskning and Kungliga Vetenskapsakademins fonder.

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Received October 14th, 1991
Accepted January 10th, 1992