Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency

G Götherström, B-A Bengtsson, I Bosæus, G Johansson and J Svensson
Research Centre for Endocrinology and Metabolism and 1Department of Clinical Nutrition, Sahlgrenska University Hospital, Göra Stråket 8, SE-41 3 45 Göteborg, Sweden

Abstract
There are few studies that have determined the effects of long-term GH replacement on bone mineral density (BMD) in GH-deficient (GHD) adults. In this study, the effects of 10 years of GH replacement on BMD were assessed in 87 GHD adults using dual energy X-ray absorptiometry (DEXA). The results show that GH replacement induced a sustained increase in BMD at all the skeletal sites measured.

Introduction: Little is known of the effect of more than 5 years of GH replacement therapy on bone metabolism in GHD adults.

Patients and methods: In this prospective, open-label, single-center study, which included 87 consecutive adults (52 men and 35 women; mean age of 44.1 (range 22–74) years) with adulthood onset GHD, the effect of 10 years of GH replacement on BMD was determined.

Results: The mean initial dose of GH was 0.98 mg/day. The dose was gradually lowered and after 10 years the mean dose was 0.47 mg/day. The mean insulin-like growth factor-I (IGF-I) SDS increased from 1.81 at baseline to 1.29 at study end. The GH replacement induced a sustained increase in total, lumbar (L2–L4) and femur neck BMD, and bone mineral content (BMC) as measured by DEXA. The treatment response in IGF-I SDS was more marked in men, whereas women had a more marked increase in the total body BMC and the total body z-score. There was a tendency for women on estrogen treatment to have a larger increase in bone mass and density compared with women without estrogen replacement.

Conclusions: Ten years of GH replacement in hypopituitary adults induced a sustained, and in some variables even a progressive, increase in bone mass and bone density. The study results also suggest that adequate estrogen replacement is needed in order to have an optimal response in BMD in GHD women.

European Journal of Endocrinology 156 55–64

Introduction
In growth hormone-deficient (GHD) adults, biochemical markers of bone turnover have demonstrated both normal (1) and decreased (2, 3) rates of bone remodeling. Histomorphometric analysis of bone in GHD men showed higher eroded surface of iliac bone and a tendency to lower osteoid surface, mineralizing surface, and bone formation rate (4). Short-term GH replacement has consistently increased biochemical markers of both bone formation and bone resorption, (5, 6) thereby suggesting increased bone remodeling.

Adults with both childhood onset (CO) and adulthood onset (AO) GHD have reduced bone mineral content (BMC) and bone mineral density (BMD) (7–10). However, the magnitude of the reduction in bone mass is more marked in adult patients with CO disease than in adult patients with AO GHD (11). In two studies, there was no detectable difference in bone mass in elderly GHD adults without GH replacement as compared with controls (12, 13). Furthermore, adult GHD patients without GH replacement therapy are at higher risk of fractures as compared with matched controls (14–16).

More than 12–18 months of GH replacement is needed to increase BMD in adult GHD of both childhood and adult onset (9, 17, 18). The responses in BMC and BMD are, however, larger in CO GHD patients than patients with AO disease (11). The effect by GH on weight-bearing regions, such as femur neck and lumbar spine, may be more prominent than the effect on other skeletal regions (9). Finally, the treatment response during GH replacement in bone mass has been more marked in GHD men than in GHD women in previous studies, lasting 2–5 years (10, 19, 20).
There are few studies determining the effects on bone mass by GH replacement ≥5 years. Five and six years respectively, of GH replacement induced a progressive increase in BMD (10, 21). In a recent 7-year study with 20 GHD patients, lumbar spine and forearm BMD increased between 1 and 6 years of GH replacement therapy and thereafter remained unchanged (22). Therefore, BMD may have reached a new plateau after 5–6 years of GH replacement therapy.

The aim of this prospective study was to investigate the effects of 10-year replacement therapy on BMD in a large group of unselected patients with adult onset GHD recruited at a single center. Furthermore, we determined clinical characteristics of patients with the largest increase in BMD and those unresponsive to GH replacement.

**Subjects and methods**

**Patients**

Eighty-seven adult patients with adult onset pituitary hormone deficiency (52 men and 35 women) and with a mean age of 44.1 (range 22–74) years were included between 1990 and 1994. All the patients had known pituitary disease or other pituitary hormonal deficiency. The pituitary deficiency was mainly caused by pituitary tumors or their treatment (Table 1). Forty-five of the patients had received only surgical treatment and thirty-one had been treated with both pituitary surgery and radiotherapy. One patient had been treated only with radiotherapy.

Most patients had multiple anterior pituitary deficiencies (Table 1). Possibly due to late effects of the radiotherapy \((n=32)\), several patients had more anterior pituitary deficiencies at the study end as compared with the baseline (Table 1). In 73 patients, the diagnosis of GH deficiency was based on a maximum peak GH response of less than 3 \(\mu g/l\) during insulin-induced hypoglycemia (blood glucose \(\leq 2.2\) mmol/l). In the remaining patients, all having multiple anterior pituitary deficiencies, the diagnosis was based on low IGF-1 values and measurements of spontaneous GH secretion \((n=12)\) or a maximum GH response <1.5 \(\mu g/l\) during a glucagon-stimulation test \((n=2)\). When required, patients received replacement therapy with glucocorticoids, thyroid hormone, gonadal steroids, and desmopressin throughout the study period. At the study start 15 out of 26 (57.7%) and at the study end 20 out of 31 (64.5%) respectively, of the gonadotropin-deficient women received estrogen replacement therapy. At study end, 3 of these women received transdermal estrogen treatment, whereas the other 17 received oral estrogen replacement. All gonadotropin-deficient men received testosterone replacement. Otherwise, the patients were not receiving any medication that could affect the measurements of this study.

Four patients died during the study (renal carcinoma \((n=1)\), cancer in the omentum (likely colonic cancer, \(n=1)\), cerebral infarction \((n=1)\), and pulmonary edema due to a probable myocardial infarction \((n=1)\)). Three patients discontinued GH therapy. One of these patients was discontinued due to lack of compliance after 8 years. The other two patients discontinued GH replacement therapy after 9.5 years, one due to a malignant tumor in the urinary bladder in combination with a cerebral infarction and one due to chronic lymphatic leukemia respectively. In addition, two patients were lost to follow-up, since they moved to other parts of Sweden. However, all the nine patients described above were retained in the statistical analysis as the last observed value for each variable was carried forward according to the intention-to-treat approach used.

**Table 1** Causes of pituitary deficiency and the type of pituitary deficiency in the study population of 87 adults with growth hormone deficiency (GHD) of adulthood onset.

<table>
<thead>
<tr>
<th>Type of deficiency</th>
<th>Men</th>
<th>Women</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated GHD</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>1 Additional deficiency</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>2 Additional deficiencies</td>
<td>9</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>3 Additional deficiencies</td>
<td>31</td>
<td>25</td>
<td>56</td>
</tr>
<tr>
<td>Diabetes insipidus</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
</tbody>
</table>

*Contains a cholesteatoma, a trauma, a meningioma.
**Study protocol**

This is an ongoing, prospective, open-label treatment trial of the administration of recombinant human GH in adult patients with GH deficiency. Eighty-seven consecutive adult patients with adulthood onset GH deficiency were treated for 10 years with GH. The initial target dose of GH in the first 64 patients was 11.9 µg/kg per day (0.25 IU/kg per week). In these 64 patients, during the first 2–3 years of treatment, the dose of GH was gradually lowered and individualized when the weight-based dose regimen was abandoned (23). In the remaining 23 patients, the GH dose was individualized from the start of the treatment.

At baseline and after each year, during the first 5 years of GH treatment, and then after 7 and 10 years, physical and laboratory examinations were performed, including measurements of bone mass and density. In addition, dose titration and safety monitoring were performed by visits every third month during the first year and every sixth month thereafter. Body weight was measured in the morning to the nearest 0.1 kg, and body height was measured barefoot to the nearest 0.01 m. No effort was made to influence the patient’s physical activity level or dietary habits during the study period.

**BMC and BMD**

Dual energy X-ray absorptiometry (DEXA) (Lunar DPX-L software version 1.3) was used to measure BMC and BMD in the total body, lumbar spine (L2–L4), and proximal femur as described previously (24). Software versions were changed several times (from 1.1 to finally 1.35) during the study, but the version 1.33 was generally used during the large period of the study. A phantom (COMAC-BME Quantitative Assessment of Osteoporosis Study Group) was frequently used for calibration purposes. The CVs between measurements were 0.4, 0.5, and 0.6% for total body BMD, lumbar (L2–L4) spine BMD, and femur neck BMD. The BMD z-score, which is the difference in S.D. of age- and sex-matched healthy subjects, and the BMD t-score, which is the difference in S.D. of sex-matched young (20–39-year old) healthy subjects, were determined using the Lunar DPX-L software program.

**Biochemical assays**

Serum IGF-I concentration was determined by a hydrochloric acid–ethanol extraction RIA using authentic IGF-I for labeling (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The individual serum IGF-I values were compared with age- and sex-adjusted values obtained from a reference population of 197 males and 195 females (25). The individual IGF-I SDS scores could then be calculated as described previously (26).

Serum osteocalcin was measured by a double-antibody RIA (International CIS, GiF-sur Yvette, France) with interassay CVs of 4.3 and 5.5% at serum concentrations of 8.9 and 19.7 µg/l respectively. Serum calcium was measured by absorption spectrophotometry (Boehringer Mannheim, Mannheim, Germany) with a total CV of ≤2.5% at a mean serum concentration of 2.5 mmol/l. Intact PTH was measured by immunoradiometric assay (Nichols Institute Diagnostics) with total CVs of 11.0, 7.5, and 6.5% at serum concentration of 13.5, 43.1, and 186 ng/l respectively. The method for determination of intact PTH was changed after 8 years of the study. The intact PTH values are therefore given only for 0–7 years of treatment.

**Statistical methods**

All the descriptive statistical results are presented as the mean (S.E.M.). For all variables, a one-way ANOVA was performed, with all data obtained from all time points, and with time as the independent variable. Post hoc analysis was performed using Student’s Newman–Keuls test. Gender differences and differences between estrogen-treated women versus women without estrogen treatment were calculated by a two-way ANOVA, with all data obtained from all time points, and with gender or estrogen treatment and time as the independent variables. In order to eliminate the baseline differences, data were transformed as percentage change or change from baseline before the between-group analyses. All analyses were performed according to the intention-to-treat principle using the last observation value carried forward principle. A two-tailed $P \leq 0.05$ was considered significant.

**Results**

**GH dose and serum IGF-I concentration**

The mean dose of GH was gradually lowered. The mean maintenance dose at 10 years was approximately half of that prescribed at the baseline (Table 2). The mean IGF-I S.D. score increased from −1.81 at the baseline to 1.29 at the study end (Table 2).

**Biochemical bone markers and BMC**

There was a sustained increase in serum concentrations of osteocalcin and calcium which were within the normal range throughout the 10 years of treatment (Table 3). Serum concentration of PTH was unchanged during 7 years of the study (Table 3). Total body, lumbar (L2–L4) spine, and femur neck BMC, as measured using DEXA, were increased from 3 to 5 years of treatment (Table 3). The increase in total BMC was progressive throughout the study period, whereas the maximum effect on lumbar (L2–L4) spine and femur neck BMC was seen after 7 years (Table 3).
Table 2 The dose of GH during 10 years of therapy in 87 growth hormone-deficient adults and the effects of this treatment on serum insulin-like growth factor-I (IGF-I), IGF-I SDS body height, body weight, and body mass index (BMI). All values are shown as the mean (S.E.M.).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 Year</th>
<th>3 Years</th>
<th>5 Years</th>
<th>7 Years</th>
<th>10 Years</th>
<th>P-value (5–10 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose of GH (mg/day)</td>
<td>0.98 (0.02)</td>
<td>0.66 (0.03)</td>
<td>0.53 (0.02)</td>
<td>0.50 (0.02)</td>
<td>0.48 (0.02)</td>
<td>0.47 (0.02)</td>
<td>0.09</td>
</tr>
<tr>
<td>IGF-I s.d. score</td>
<td>-1.81 (0.12)</td>
<td>3.10 (0.29)</td>
<td>5.25 (0.25)</td>
<td>1.88 (0.24)</td>
<td>1.51 (0.19)</td>
<td>1.29 (0.25)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum IGF-I (μg/l)</td>
<td>99.5 (6.6)</td>
<td>341.1 (14.1)</td>
<td>303.0 (12.0)</td>
<td>281.0 (13.0)</td>
<td>279.6 (14.5)</td>
<td>223.3 (9.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>172.9 (1.1)</td>
<td>173.3 (1.2)</td>
<td>173.5 (1.2)</td>
<td>173.3 (1.2)</td>
<td>173.1 (1.2)</td>
<td>172.7 (1.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>82.0 (1.8)</td>
<td>82.3 (1.9)</td>
<td>83.4 (1.8)</td>
<td>84.1 (1.8)</td>
<td>84.2 (1.8)</td>
<td>83.9 (1.7)</td>
<td>0.92</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8 (0.7)</td>
<td>27.3 (0.5)</td>
<td>27.7 (0.5)</td>
<td>27.9 (0.5)</td>
<td>28.0 (0.7)</td>
<td>28.1 (0.7)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

The statistical analyses are based on a one-way ANOVA followed by Student’s Newman–Keuls post hoc test. *P < 0.05 †P < 0.01 ‡P < 0.001 (for the dose of GH: vs initial GH dose; for other variables: vs baseline).

BMD

Total BMD and t-score decreased initially and transiently by the GH replacement and after 7 and 10 years of treatment, total BMD, z-, and t-scores increased as compared with the baseline (Table 4). Lumbar (L2–L4) BMD, t- and z-scores increased throughout the study with maximum effect after 10 years (Table 4). Femur neck BMD and z-score achieved a peak value between 5 and 7 years of the study (Table 4). At the baseline, a large group of patients had osteopenia and some of them met the criteria for osteoporosis (t-score less than −2.5 s.d.) in total body, lumbar (L2–L4) spine, and femur neck t-score (Table 5). In 37–55% of the patients, t-score increased after 10 years (Table 5). The share of the patients with z-score >0 increased with 12% in total body BMD, 27% in lumbar (L2–L4) spine BMD, and 20% in femur neck BMD after 10 years (Fig. 1A–C).

Responders versus non-responders in BMD

The patients who received high doses of GH based on body weight at initiation of treatment had a similar response after 10 years in all measures of BMC and BMD as compared with patients who received individualized, low-dose GH replacement from the beginning (data not shown).

An analysis of responders versus non-responders was performed in terms of femur neck BMD as it was at this skeletal site that most patients had osteopenia at the baseline (Table 5). The patients with remaining osteopenia after 10 years (n = 20) were overall older (mean age 64.2 (2.2), vs 57.1 (1.4) years respectively, P < 0.05) and included a larger proportion of women (59 vs 33.3% respectively, P < 0.05).

The patients who decreased in femur neck BMD t-score at 10 years (approximately 12%) showed no difference in age, sex, diagnosis, and number of pituitary deficiencies or frequency of estrogen replacement when compared with the patients (37%) who increased in femur neck BMD t-score.

Patients with a history of secreting pituitary adenomas (n = 18) as well as the subgroup of patients with a history of prolactinomas (n = 12) had a more marked response to GH replacement after 10 years in terms of femur neck z-score (both P = 0.05 vs patients with a history of non-secreting pituitary adenomas) and femur neck t-score (both P < 0.05). In all other measures of BMC and BMD, the response after 10 years was similar as that in patients with non-secreting pituitary adenomas (data not shown). The responsiveness to GH replacement could not be evaluated in patients with previous Cushing’s disease or acromegaly due to the small number of patients in these groups (n = 4 and n = 2 respectively).

Fractures

No patient had any fracture during the study period.

Gender differences

The dose of GH (mg/day) was similar in both sexes. Adjusted for body weight, however, the mean dose of GH was higher in women than in men at all time-points of the study except for the dose prescribed at the baseline visit (at study end the dose was 6.5 (0.8) μg/kg per day in women vs 6.0 (0.8) μg/kg per day in men).

Table 3 Effects of 10 years of growth hormone (GH) substitution in 87 GH-deficient adults on serum concentrations of biochemical bone markers as well as on bone mass as determined using dual energy X-ray absorptiometry. All values are shown as the mean (S.E.M.).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 Year</th>
<th>3 Years</th>
<th>5 Years</th>
<th>7 Years</th>
<th>10 Years</th>
<th>P-value (5–10 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin (μg/l)</td>
<td>9.2 (0.3)</td>
<td>14.8 (0.5)</td>
<td>11.4 (0.4)</td>
<td>11.9 (0.4)</td>
<td>11.9 (0.4)</td>
<td>12.9 (0.5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>2.27 (0.01)</td>
<td>2.32 (0.01)</td>
<td>2.30 (0.01)</td>
<td>2.31 (0.01)</td>
<td>2.36 (0.01)</td>
<td>2.35 (0.01)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intact PTH (ng/l)</td>
<td>39.6 (1.8)</td>
<td>37.3 (3.9)</td>
<td>43.3 (2.2)</td>
<td>44.2 (2.1)</td>
<td>43.7 (2.2)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Total body BMC (kg)</td>
<td>2.74 (0.08)</td>
<td>80.0 (0.08)</td>
<td>82.3 (0.08)</td>
<td>82.2 (0.08)</td>
<td>82.2 (0.08)</td>
<td>82.2 (0.08)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lumbar (L2–L4) BMC (g)</td>
<td>55.4 (3.4)</td>
<td>56.1 (3.4)</td>
<td>58.5 (3.8)</td>
<td>59.6 (3.8)</td>
<td>60.2 (2.1)</td>
<td>60.1 (2.4)</td>
<td>0.23</td>
</tr>
<tr>
<td>Femur neck BMC (g)</td>
<td>5.03 (0.17)</td>
<td>5.01 (0.14)</td>
<td>5.16 (0.15)</td>
<td>5.26 (0.17)</td>
<td>5.33 (0.14)</td>
<td>5.21 (0.13)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

PTH, parathyroid hormone; BMC, bone mineral content. The statistical analyses are based on a one-way ANOVA followed by Student’s Newman–Keuls post hoc test. *P < 0.05 †P < 0.01 ‡P < 0.001 (for the dose of GH: vs initial GH dose; for other variables: vs baseline).
Table 4 Effects of 10 years of growth hormone (GH) substitution in 87 GH-deficient adults on bone mineral density (BMD). All values are shown as the mean (s.e.m.).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 Year</th>
<th>3 Years</th>
<th>5 Years</th>
<th>7 Years</th>
<th>10 Years</th>
<th>P-value (5–10 year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEXA total body</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD (g/cm²) z-score</td>
<td>1.163 (0.015)</td>
<td>1.155 (0.014)</td>
<td>1.165 (0.014)</td>
<td>1.162 (0.015)</td>
<td>1.178 (0.015)</td>
<td>1.194 (0.016)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>t-score</td>
<td>−0.27 (0.12)</td>
<td>−0.34 (0.11)</td>
<td>−0.28 (0.12)</td>
<td>−0.27 (0.12)</td>
<td>−0.03 (0.13)</td>
<td>0.21 (0.15)</td>
<td></td>
</tr>
<tr>
<td>DEXA lumbar (L2–L4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD (g/cm²) z-score</td>
<td>1.161 (0.022)</td>
<td>1.166 (0.023)</td>
<td>1.210 (0.024)</td>
<td>1.227 (0.026)</td>
<td>1.235 (0.026)</td>
<td>1.243 (0.029)</td>
<td>0.31</td>
</tr>
<tr>
<td>t-score</td>
<td>−0.16 (0.16)</td>
<td>0.12 (0.18)</td>
<td>0.18 (0.17)</td>
<td>0.37 (0.18)</td>
<td>0.41 (0.19)</td>
<td>0.54 (0.21)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>DEXA femur neck</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD (g/cm²) z-score</td>
<td>0.939 (0.017)</td>
<td>0.949 (0.017)</td>
<td>0.973 (0.016)</td>
<td>0.977 (0.017)</td>
<td>0.978 (0.017)</td>
<td>0.976 (0.017)</td>
<td>0.80</td>
</tr>
<tr>
<td>t-score</td>
<td>−0.29 (0.11)</td>
<td>−0.15 (0.11)</td>
<td>0.07 (0.11)</td>
<td>0.18 (0.11)</td>
<td>0.24 (0.12)</td>
<td>0.19 (0.13)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

DEXA, dual energy X-ray absorptiometry. The statistical analyses are based on a one-way ANOVA followed by Student–Newman–Keuls post hoc test. P-values (5–10 years) are based on the post hoc analyses between the 5-year and 10-year values.

Women on estrogen vs women without estrogen

At study start, 15 out of 26 (57.7%) and at study end, 20 out of 31 (64.5%) respectively, of the gonadotropin-deficient women received estrogen replacement therapy. Women on estrogen replacement and eugonadal women (n=24) had a non-significant tendency to a higher increase in lumbar (L2–L4) spine t-score BMD (P=0.09), femur neck BMD (P=0.07), and total BMD (P=0.07) as compared with gonadotropin-deficient women without estrogen treatment (n=11). Estrogen-replete women had a higher mean GH dose throughout the study than hypogonadal women without estrogen therapy (P<0.05, data not shown).

If a comparison is made between women receiving estrogen substitution at the study (n=21) end versus women who did not (n=14), there will be a non-significant tendency to a higher percentage increase in the total body BMD in women receiving estrogen treatment (Fig. 3A). The percentage change in total BMC (Fig. 3B) and BMD lumbar (L2–L4) spine t-score level (Fig. 3C) were significantly higher in women on estrogen replacement therapy. There was no difference in GH dose, IGF-I, and bone formation markers between these two groups. Baseline age tended to be higher in women on estrogen treatment than those who did not, but the difference was not statistically significant (the mean age 50.5 (2.4) vs 48.3 (3.8) years respectively, P=NS). Furthermore, the results of all analyses between women on estrogen treatment versus women without estrogen treatment also remained when the baseline age was used as the covariant.

The possible importance of the effect of initiation of estrogen substitution during the study period could not be statistically evaluated because of the relatively small number of women in this group (n=5).

GHD women above 55 years of age had a less marked response to GH replacement in relation to women below 55 years in terms of total body BMD (P<0.05) and total body BMC t-score (P<0.05). However, the response in femur neck t-score was more marked in the women above 55 years of age (P<0.01). In other variables

Table 5 Osteopenia and osteoporosis at baseline and after 10 years of growth hormone (GH) treatment in 87 GH deficient adults according to t-score s.d.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (%)</th>
<th>After 10 years (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Osteopenia (t-score)</td>
<td>Osteoporosis (t-score)</td>
</tr>
<tr>
<td></td>
<td>–1.0 s.d.</td>
<td>–2.5 s.d.</td>
</tr>
<tr>
<td>Total BMD</td>
<td>31.9</td>
<td>8.5</td>
</tr>
<tr>
<td>Lumbar (L2–L4) BMD</td>
<td>25.5</td>
<td>12.8</td>
</tr>
<tr>
<td>Femur Neck BMD</td>
<td>46.8</td>
<td>4.4</td>
</tr>
</tbody>
</table>

BMD, bone mineral density.
Figure 1 Percentage of the 87 patients (52 men and 35 women) with adult onset GH deficiency having bone mineral density z-score > 0 in (A) total body, (B) lumbar (L2–L4) spine, and (C) femur neck, estimated by dual energy X-ray absorptiometry, during 10 years of GH replacement.

Figure 2 The percentage change in (A) total body bone mineral content (BMC), and (B) total body, (C) lumbar (L2–L4) spine, and (D) femur neck bone mineral density (BMD) estimated by dual energy X-ray absorptiometry in men and women during 10 years of GH replacement in 87 patients with adult onset GH deficiency. The vertical bars indicate the 95% confidence interval (CI) for the mean values shown. Between-group P values are based on an analysis of the percentage change from the baseline whereas within-group P values are based on an analysis of the absolute values. *P<0.05; †P<0.01; ‡P<0.001 (vs baseline).
Effects of 10-year growth hormone (GH) replacement in 52 GH deficient (GHD) men and 35 GHD women on bone mineral content (BMC) and bone mineral density. All values are shown as the mean (S.E.M.).

<table>
<thead>
<tr>
<th>Gender Baseline 1 Year 3 Years 5 Years 7 Years 10 Years (5–10 years) P-value</th>
<th>Between-group P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total body</strong></td>
<td>0.17 (0.13)</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>0.36 (0.21)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>0.27 (0.15)</td>
</tr>
<tr>
<td><strong>Baseline Z-Score (S.D.) Women</strong></td>
<td>0.06 (0.17)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Baseline BMC (g) Women</strong></td>
<td>63.2 (2.0)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>62.2 (2.0)</td>
</tr>
<tr>
<td><strong>Baseline Z-Score (S.D.) Women</strong></td>
<td>0.00 (0.17)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Baseline BMC (g) Women</strong></td>
<td>47.1 (2.1)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>57.7 (1.4)</td>
</tr>
<tr>
<td><strong>Baseline Z-Score (S.D.) Women</strong></td>
<td>0.00 (0.17)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Baseline BMC (g) Women</strong></td>
<td>4.09 (0.20)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>4.09 (0.20)</td>
</tr>
<tr>
<td><strong>Baseline Z-Score (S.D.) Women</strong></td>
<td>0.00 (0.17)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Baseline BMC (g) Women</strong></td>
<td>4.09 (0.20)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>4.09 (0.20)</td>
</tr>
<tr>
<td><strong>Baseline Z-Score (S.D.) Women</strong></td>
<td>0.00 (0.17)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>0.001</td>
</tr>
</tbody>
</table>

The between-group differences are based on an analysis of the percent change from baseline. *P < 0.05, **P < 0.01, ***P < 0.001 (vs baseline) NS indicates not significant.

Discussion

This single-center study is the largest and longest observational study of the long-term effects of GH replacement therapy in hypopituitary adults with adult onset GHD on bone mass and density. The results show that the 10-year GH replacement therapy produced sustained increase in bone mass and density with the maximum effect after 7–10 years of treatment.

The dose of GH, which was based on body weight at the initiation of treatment in most patients, was gradually lowered and individualized. The increase in serum concentrations of osteocalcin and calcium were, however, sustained suggesting increased bone turnover and increased intestinal uptake of calcium to be of important for the increased bone mass and density in response to GH. In support of this, supraphysiological doses of GH favored the production of 1,25(OH)2D3 reflecting bone mass and density, the treatment response was similar in both groups.

**Correlation analysis**

Baseline femur neck BMC correlated inversely with the percentage change in the same variable ($r = -0.31$, $P = 0.01$). There was no correlation between the baseline values and the percentage change in total body or lumbar (L2–L4) BMC ($r = 0.15$ and $r = 0.09$ respectively). This indicates that those patients with the lowest the baseline values in the femur neck BMC had the greatest increase in this variable, whereas no such relation was observed for total body and lumbar (L2–L4) spine BMC.

There was a positive correlation between the age at the baseline and the percentage change in the lumbar (L2–L4) spine BMC ($r = 0.26$, $P < 0.05$), suggesting that the oldest patients had the largest increase in lumbar (L2–L4) spine BMC. No such correlation was found between age and the other skeletal sites (data not shown).

There was a negative correlation between the baseline serum IGF-I and percentage change femur neck BMC ($r = -0.26$, $P < 0.05$), and a positive correlation between the percentage changes in the variables after 10 years ($r = 0.30$, $P < 0.05$). No other correlations were found between serum IGF-I and BMC and BMC values, neither at the baseline nor between the percentage changes after 10 years.

There was no positive correlation between the response in total, lumbar (L2–L4) spine, or femur neck BMC after 1 year and the response in the same variable between 1 and 10 years of GH replacement (data not shown). The patients with a good treatment response after 1 year may therefore not be the same patients as those who have a good treatment response in BMC after 10 years.
over that of 24,25(OH)2D3 with a moderate increase in intestinal Ca absorption in dogs (27). In a study in GHD humans, GH administration at bedtime caused a nocturnal increase in PTH production followed by a decrease in PTH during daytime as compared with untreated GHD adults (28). It could be hypothesized that such a 24-h PTH pattern during GH therapy could be beneficial for bone density.

In agreement with several shorter studies (29, 30), an initial transient decrease in total body BMD occurred during the first year of GH replacement. Total body BMC and lumbar (L2–L4) spine z-score increased progressively between years 5 and 10 of the study, whereas the maximum effect on lumbar (L2–L4) spine BMC, and femur neck BMC and BMD, was observed after approximately 7 years of GH replacement therapy. Studies with longer duration than the present one have to evaluate if the maximum effect of GH on bone mass and density has been reached after 10 years of therapy or if BMC and BMD can increase even more during prolonged GH replacement therapy.

T-scores were almost normalized by the GH replacement therapy. Since t-score in lumbar spine and femur neck is strongly related to the risk of fractures in these regions (31, 32), the 10-year GH replacement is likely to reduce the risk of fractures in GHD adults. GH replacement can also normalize muscle strength (33, 34) which together with a possible increase in physical activity could explain the increase in BMD by GH replacement. Furthermore, the analysis of patients who increased their t-score at the femur neck versus those who decreased in femur neck t-score showed no clear difference in the baseline characteristics between these two groups of patients. After 10 years, the patients with remaining osteopenia at the femur neck were overall older and included a larger proportion of women than the patients without osteopenia at this skeletal site. This is also in line with that observed in the normal population and suggests that most individuals with low BMD are elderly women, in hypopituitary adults as well as in the normal population.

Testosterone replacement improves BMD in hypogonadal men (35). In the present study, all hypogonadal men received testosterone replacement. The testosterone replacement may therefore have contributed to the increase in BMD observed in men. However, in contrast to most previous studies where women were less responsive to GH replacement (20, 36, 37), women in our study showed, after 10 years, an outcome in bone mass and density at least similar to that of men. The increase in total body BMC and z-score were even more marked in women than in men.

The prominent effect on BMD in women in this study could be a combined effect of the GH and the estrogen replacement during the long period of time. In postmenopausal women, oral estrogen treatment has been shown to lower serum IGF-I concentration (38) and increase BMD (39). Studies in transgenic mice show that GH can only increase bone in female mice with adequate estrogen levels (40). In the present study, women on estrogen therapy at the end of the 10-year study had a tendency to greater increase in total BMC and lumbar (L2–L4) spine z-score than women without estrogen therapy. Furthermore, women who increased their femur neck BMC had estrogen treatment to a higher extent than women who decreased femur neck BMD at the study end as compared with the baseline. Although the group of women who initiated estrogen replacement therapy had a higher risk score of osteoporosis, the increase in BMD was not significantly different compared with women without estrogen therapy.
GH replacement in hypopituitary patients

 References


Acknowledgements

This study was supported by the chair of Göteborg, Novo Nordisk and Pfizer. We are indebted to Lena Wiren, Ingrid Hansson, Sigrid Lindstrand, Anna-Lena Jönsson, and Annika Alkind at the Research Centre for Endocrinology and Metabolism Sahlgrenska University Hospital in Göteborg, for their skillful technical support.

treatment during the GH replacement was not large enough to be statistically evaluated. Increased frequency of estrogen replacement could have induced, or been permissive for, the prominent increase in BMD in the GH women.

Previous studies suggest that estrogen may have a more profound effect on bone remodeling than testosterone (41), with an effect on both bone resorption and bone formation. We cannot exclude that gender-dependent differences in bone turnover may result in that an increase in bone mass by a GH-induced increase in bone turnover is detected slower in women. However, in a study with as long duration as the present one, gender-dependent changes due to differences in bone remodeling rate are probably minimized. An increase in bone mass by increased bone turnover by GH may therefore first be detected in men.

This study shows that GH replacement is of substantial benefit for bone in hypopituitary men and women with adult onset GHD. To what extent GH treatment is of value in osteoporotic patients without severe GHD is not fully clear. Untreated adult GHD is probably a state of low bone turnover (4), whereas in osteoporosis, there is an uncoupling of bone resorption versus bone formation, resulting in increased bone resorption (42, 43). In a placebo-controlled study of osteoporotic postmenopausal women on estrogen treatment, GH treatment resulted in marked increases in total, lumbar spine, femur neck, and BMC after 4 years, that is, 1 year after discontinuation of GH treatment (44). In an open study in osteoporotic men with calcium and vitamin D supplementation, continuous 2-year GH treatment increased lumbar spine and total body BMD as compared with the baseline (45, 46). However, there is a need for further studies, which will preferably also include fracture data, before more firm conclusions can be made regarding the usefulness of GH treatment in osteoporotic patients without severe GHD.

In conclusion, 10 years of GH replacement in adults with adult onset GHD induced a sustained, and in some variables even a progressive, increase in bone mass and bone density. This increase in BMC and BMD was seen both in the GHD men and women. Adequate estrogen replacement in hypopituitary women augments the increase in bone mass and density in response to GH replacement.
hormone replacement therapy, and other aspects of hypopituitary
tissue on fracture rate and bone mineral density. Bone and Mineral
16 Wuster C. Fracture rates in patients with growth hormone
17 Degerblad M, Eldingy N, Hall K, Sjoberg H & Thoren M. Potent
effect of recombinant growth hormone on bone mineral density
18 Vandeweghe M, Taelman P & Kaufman J. Short and long-term
effects of growth hormone treatment on bone turnover and bone
mineral content in adult growth hormone-deficient males. Clinical
19 Drake WM, Rodriguez-Arnau J, Weaver IU, James IT, Coyle D,
Spector TD, Besser GM & Monson JP. The influence of gender
on the short and long-term effects of growth hormone replacement
on bone metabolism and bone mineral density in hypopituitary
20 Johansson AG, Engstrom BE, Ljunghall S, Karlsson FA &
Burman P. Gender differences in the effects of long term growth
hormone (GH) treatment on bone in adults with GH deficiency.
21 Cianget C, Seck T, Hinke V, Wuster C, Ziegler R & Pfilschifter J.
Effects of 6 years of growth hormone (GH) treatment on bone
22 Wilhelm B & Kann PH. Long-term effects of 7-year growth
hormone substitution on bone metabolism, bone density, and bone
quality in growth hormone-deficient adults. Medizinische Klinik
23 Johansson G, Rosén T & Bengtsson B-A. Individualized dose
titrolation of growth hormone (GH) during GH replacement in
24 Mazess R, Barden H, Bisek J & Hanson J. Dual-energy X-ray
absorptiometry for total-body and regional bone-mineral and soft-
1106–1112.
25 Landin-Wilhelmsen K, Wilhelmsen L, Lappas G, Rosén T,
Lindstedt G, Lundberg P-A & Bengtsson B-A. Serum insulin-like
growth factor I in a random population sample of men and
women: relation to age, sex, smoking habits, coffee consumption
and physical activity, blood pressure and concentrations of plasma
lipids, fibrinogen, parathyroid hormone and osteocalcin. Clinical
Endocrinology 1994 41 351–357.
26 Svensson J, Johansson G & Bengtsson B-A. Insulin-like growth
factor-I in growth hormone-deficient adults: relationship to
population-based normal values, body composition and insulin
27 Tryfonidou MA, Holl MS, Oosterlaken-Dijksterhuis MA,
Vastenburg M, Van Den Brom WE & Hazewinkel HA. Growth
hormone modulates cholecalciferol metabolism with moderate
effects on intestinal mineral absorption and specific effects on bone
formation in growing dogs raised on balanced food. Domestic
28 Ahmad AM, Thomas J, Clewes A, Hopkins MT & Guzder R. Effects
of growth hormone replacement on parathyroid hormone sensitivity
and bone mineral metabolism. Journal of Clinical Endocrinology
and Metabolism 2003 88 2860–2868.
29 Cuneo RC, Judd S, Wallace JD, Perry-Keene D, Burger H, Lim-Tio S,
Strauss B, Stockigt J, Topliss D, Alford F, Hew L, Bode H, Conway A,
Handelsman D, Dunn S, Boyages S, Cheung NW & Hurley D. The
Australian multicenter trial of growth hormone (GH) treatment in
30 Johansson G & Ohlsson C. Growth hormone therapy and fracture
risk in the growth hormone-deficient adult. Baillieres Clinical
31 Cummings S, Black D, Nevitt M, Browner W, Cauley J, Ensrud K,
Genant H, Palermo L, Scott J & Vogt T. Bone density at various sites
32 Melton III L, Atkinson E, O’Fallon W, Wahner H & Riggs B. Long-
term fracture prediction by bone mineral assessed at different
Two years of growth hormone (GH) treatment increase isometric and
isokinetic muscle strength in GH-deficient adults. Journal of Clinical
Endocrinology and Metabolism 1997 82 2877–2884.
34 Svensson J, Stirbrant Sonnerhagen K & Johansson G. Five years of
growth hormone replacement therapy in adults: age and gender-
related changes in isometric and isokinetic muscle strength. Journal of Clinical Endocrinology and Metabolism 2003 88
2061–2069.
35 Benito M, Vasilec B, Wehrli FW, Bunker B, Wald M, Gomberg B,
Wright AC, Zemel B, Cuccia A & Snyder PJ. Effect of testosterone
replacement on trabecular architecture in hypogonadal
36 Volmaki MJ, Salmela PL, Salmi J, Vikari J, Kataja M, Turunen H &
Soppi E. Effects of 42 months of GH treatment on bone mineral
density and bone turnover in GH-deficient adults. European
37 Bex M, Abs R, Maiter D, Beckers A, Lambertigs G & Bouillon R. The
effects of growth hormone replacement therapy on bone metabolism in adult-onset growth hormone deficiency: a 2-year
open randomized controlled multicenter trial. Journal of Bone and
38 Ho KK, O’sullivan AJ, Wothers T & Leung KC. Metabolic effects of
39 Pepi TWCFT. Effects of hormone therapy on bone mineral density:
results from the postmenopausal estrogen/progestin interventions
(PEPI) trial. JAMA 1996 276 1389–1396.
40 Sandstedt J, Tornell J, Norjarava E, Isaksson OG & Ohlsson C.
Evaluated levels of growth hormone increase bone mineral content
41 Falahati-Nini A, Riggs BL, Atkinson EJ, O’fallon WM, Eastell R &
Khosla S. Relative contributions of testosterone and estrogen in
regulating bone resorption and formation in normal elderly men.
42 Heaney RP, Recker Rr & Saville Pd. Menopausal changes in
boneremodeling. Journal of Laboratory and Clinical Medicine 1978
92 953–963.
43 Parfit AM, Matthews Ce, Villanueva Ar, Kleerekoper M, Frame B
& Rao Ds. Relationships between surface, volume and thickness of
iliac trabecular bone in aging and in osteoporosis. Journal of
Clinical Investigation 1983 72 1396–1409.
44 Landin Wilhelmsen K & Nilsson A. Growth hormone increases
bone mineral content in postmenopausal osteoporosis. Journal of
45 Nilsson AG. Effects of growth hormone replacement therapy on
bone markers and bone mineral density in growth hormone-
S & Nilsson A 2001 Increased bone density following two years of
growth hormone(GH) treatment in men with idiopathic osteo-
porosis. 83rd Annual Meeting of the Endocrine Society, Denver,
USA. Abstract OR42-4.

Received 12 July 2006
Accepted 11 October 2006

www.eje-online.org