VU Research Portal

Effects of 10 years of growth hormone (GH) replacement therapy in adult GH-deficient men

Arwert, Lucia I; Roos, Jan C; Lips, Paul; Twisk, Jos W R; Manoliu, Radu A; Drent, Madeleine L

published in

Clinical Endocrinology
2005

DOI (link to publisher)

[10.1111/j.1365-2265.2005.02343.x](https://doi.org/10.1111/j.1365-2265.2005.02343.x)

document version

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

citation for published version (APA)

Arwert, L. I., Roos, J. C., Lips, P., Twisk, J. W. R., Manoliu, R. A., & Drent, M. L. (2005). Effects of 10 years of growth hormone (GH) replacement therapy in adult GH-deficient men. *Clinical Endocrinology*, 63(3), 310-316. <https://doi.org/10.1111/j.1365-2265.2005.02343.x>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

vuresearchportal.ub@vu.nl

ORIGINAL ARTICLE

Effects of 10 years of growth hormone (GH) replacement therapy in adult GH-deficient men

Lucia I. Arwert*, Jan C. Roost†, Paul Lips*, Jos W. R. Twisk‡, Radu A. Manoliu† and Madeleine L. Drent*

Departments of *Endocrinology, †Radiology and ‡Clinical Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, the Netherlands

Summary

Objective GH-deficient adults have changes in body composition, bone mineral density (BMD) and lipid profile that can be altered by GH substitution. However, long-term data on GH substitution (up to 10 years of follow-up) are limited.

Design The effects of 10 years of GH replacement therapy on BMD, body composition, bone parameters, serum lipids and glucose metabolism were studied.

Patients Twenty-three childhood-onset GH-deficient men (mean age at baseline 28.6 years) were studied during 10 years of GH substitution therapy. A group of 19 age-matched healthy men served as a control group for BMD measurements at baseline and after 10 years.

Results BMD of the lumbar spine increased during the 10 years of GH therapy. Bone markers and BMD in the hip increased during the first 5 years of GH therapy, but were not different from baseline after 10 years. BMD changes over time in the lumbar spine and femoral neck were significantly different in the patients compared to the controls. After 10 years the difference between the groups had decreased, but BMD was still higher in the controls than in the patients. Lipid profile had improved after 10 years of GH therapy, but body mass index (BMI), waist–hip ratio (WHR), fasting glucose and glycosylated haemoglobin (HbA1c) had increased compared to baseline.

Conclusions This long-term follow-up study found that 10 years of GH substitution in GH-deficient men causes sustained improvements in BMD in the lumbar spine and lipid profile but not in body composition.

(Received 14 February 2005; returned for revision 9 March 2005; finally revised 31 March 2005; accepted 7 June 2005)

Introduction

The major role of GH during childhood is to promote longitudinal bone growth and linear growth, but GH continues to have important metabolic actions throughout life.¹ Besides growth, GH is known to affect body composition, bone mineralization, and lipid and glucose metabolism. In adults, the condition of GH deficiency (GHD) has been accepted as a well-defined clinical syndrome.² The most apparent symptoms include altered body composition (increased fat mass, decreased lean body mass, decreased muscle mass), decreased bone mineral density (BMD), altered lipid profile, reduced sense of psychological well-being, and impaired cognitive functioning.³ Replacement with recombinant human GH (rhGH) for several years has been shown to ameliorate these impairments.^{4–6}

The mechanism of decreased BMD in GH-deficient patients is probably related to reduced bone formation.⁷ Reduced BMD has been reported in patients with childhood-onset GHD (CO-GHD)⁸ and adulthood-onset GHD (AO-GHD),⁹ both in isolated GHD (IGHD) and in multiple pituitary hormone deficiency (MPHD).^{10,11} A meta-analysis of the short-term effect of GH replacement on BMD demonstrated a small effect of GH replacement on BMD in the lumbar spine.¹² The BMD in the lumbar spine increased by 0.01 g/cm² after 12 months of GH treatment, 0.02 g/cm² after 18 months, and 0.03 g/cm² after 24 months. Several studies have shown that bone mass and lean body mass continue to increase after epiphyseal fusion.^{7,13}

In a recent meta-analysis of the short-term effects of GH treatment on cardiovascular risk factors, it was shown that GH substitution with a maximum duration of 18 months resulted in reduced low density lipoprotein (LDL)-cholesterol, total cholesterol, fat mass, and diastolic blood pressure. Lean body mass, fasting plasma glucose, and increased insulin increased significantly.¹⁴

Long-term experience of the effects of GH substitution in GH-deficient adults is limited. To date, only one study of GH replacement in adults with GHD over a period up to 10 years has been published.¹⁵ In this study, GH treatment for 10 years in GH-deficient adults resulted in an increase of lean body mass and muscle mass, a less atherogenic lipid profile, reduced carotid intima media thickness, and improved psychological well-being. However, BMD after 10 years follow-up was not measured and only 10 GH-deficient adults were followed in the study.

Correspondence: Lucia I. Arwert, Department of Endocrinology, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, the Netherlands. Tel.: +31 20 4442799; Fax: +31 20 4440502; E-mail: l.arwert@vumc.nl

In an earlier study from our institution, the effect of GH on body composition, BMD and cardiac function was measured after 5 years of treatment of men with CO-GHD.¹⁶ The present study evaluates the effects of 10 years of GH replacement therapy on body composition, BMD, bone markers, lipid profile and glucose metabolism in a group consisting of 23 adult men with CO-GHD. Results for the BMD in patients after 10 years of GH substitution were compared to longitudinal BMD changes in age- and sex-matched healthy men to control for natural changes in BMD associated with ageing.

Subjects and methods

Study design

This study started as a 6-month double-blind, placebo-controlled trial followed by an open extension period of GH therapy for 5 years.^{10,16} For the placebo group, the starting point of the GH substitution was at 6 months. GH substitution continued for at least 10 years and study observations were made at baseline, regularly during the first 5 years, and for the present study after 10 years of GH replacement therapy. All patients were seen regularly in the outpatient clinic during the 10 years of follow-up to check for adequate hormone substitution. The study is restricted to data from those subjects who completed the entire 10-year period of continuous GH treatment.

Subjects

Fifty adult men with CO-GHD were included at the start of the study as described previously.¹⁷ This patient group was chosen to study a homogeneous group and to prevent bias by sex-related differences, by associated medical disorders or medication. All these patients had received GH replacement for several years during childhood and were retested for GHD before inclusion. Inclusion criteria for the study were: (1) discontinuation of previous GH treatment for short stature for at least 1 year; (2) a serum IGF-I concentration of at least 2 SD below the age-related normal mean and a maximal GH response to 100 µg/kg GHRH or insulin-induced hypoglycaemia (using an insulin tolerance test, ITT) of less than 7 µg/l; and (3) stable conventional hormone replacement for patients with MPHD. Twelve patients were excluded from the study during the first years, as described previously,¹⁶ and 38 patients were studied for a follow-up period of 39–69 months. Of these 38 patients, 13 discontinued their GH therapy between 5 and 10 years follow-up for different reasons (10 withdrew due to lack of motivation and noncompliance, two patients discontinued due to adverse events (one patient developed hypertension and the other complained of sleeping difficulties) and one patient discontinued because of anxiety of needles. Only the patients still treated with GH ($n = 25$) were asked to participate in the present long-term, follow-up study. One patient moved abroad and one patient refused to participate. The remaining 23 patients were included in this 10-year follow-up study (17 patients with MPHD and six patients with IGHD). Three patients were treated for craniopharyngioma during childhood, 11 patients were diagnosed with empty sella syndrome, and nine patients had idiopathic GHD. The patients with MPHD received stable hormone replacement:

thyroid hormone ($n = 16$, mean dose 124.2 ± 23.5 µg/day), cortisone-acetate ($n = 14$, mean dose 25.2 ± 5.9 mg/day), testosterone substitution ($n = 12$), human gonadotrophins ($n = 3$) and antidiuretic hormone ($n = 2$). The first 6 months of the study were placebo-controlled (placebo or a GH dose of 1, 2 or 3 U/m² per day), thereafter the GH dose was fixed at 2 U/m².¹⁷ After 2 years the GH dose was titrated based on serum IGF-I levels in the normal range (for age and sex).

Fifty-six, healthy, age-matched men served as a control group for BMD assessments at the start of the study¹⁰ and were asked to participate again 10 years later. Twenty-five subjects of the control group at the start of the study were tracked down after 10 years. Nineteen volunteers were included in the present study, three control subjects refused to participate, and three control subjects could not participate for logistic reasons. Written informed consent was obtained at the start of the study and again before the 10-year follow-up test procedure started. The study was approved by the Medical Ethical Committee of the VU University Medical Centre and conducted according to the principles of the Helsinki Declaration.

Study parameters

BMD measurements

BMD (lumbar spine, femoral neck, trochanter) was measured by dual-energy X-ray absorptiometry (DEXA). In the GH-deficient patients and control subjects DEXA scans of the lumbar spine (L2–L4) and the nondominant hip (femoral neck, trochanter) were obtained by standard procedures supplied by the manufacturer. BMD was calculated by the software program and presented as the areal density expressed in grams per cm².

During the first 5 years of the study the Norland XR-26 was used as described previously.¹⁶ The long-term precision of the device (coefficient of variation of repeated measurements after 4 months, assessed in a group of normal subjects and in osteoporotic subjects) was 2.1% for the femoral neck and 2.4% for the femoral trochanter.¹⁸

BMD measurements after 10 years of GH substitution were carried out using the Hologic QDR-4500 instrument, which had replaced the Norland XR-26 device. To control for possible differences within and between the two devices, BMD measurements on the European Spine Phantom (ESP) were performed on both devices. The mean BMD of the ESP phantom measured on the Norland XR-26 was 0.94 ± 0.004 g/cm² and on the Hologic-4500 0.92 ± 0.01 g/cm². We also analysed the changes in BMD after 10 years in the GH-deficient group and in the control group to control for differences in both instruments and for time effects with normal ageing.

Body composition

In the patients with GHD, body composition was assessed by anthropometric measurements (body mass index (weight/height²), waist, hip, upper arm and leg circumference and triceps skin-fold measurements). Measurements were performed as described previously.¹⁹

Fig. 1 Bone mineral density in the lumbar spine (L2–L4), and femoral neck and trochanter at baseline and during follow-up in the GH-deficient patients.

Laboratory investigations

Blood samples were taken in the fasting state in the GH-deficient patients. The serum IGF-I concentration during the first 5 years of this study was measured by radioimmunoassay (RIA, Medgenics Diagnostics, Fleurus, Belgium) after acid–ethanol extraction of IGF binding proteins. Intra- and interassay coefficients of variation (CVs) were 6 and 10%, respectively. The IGF concentration after 10 years was measured by a chemoluminescent assay (Nichols Institute Diagnostics, San Juan Capistrano, USA). The intra-assay CV is 3% and the interassay CV is 6%. Serum osteocalcin concentrations were analysed by RIA (Incstar Corporation, Stillwater, USA). Urinary calcium, creatinine and hydroxyproline excretion were assessed in a 2-h urine morning sample, collected between 0800 and 1000 h as described previously.²⁰ Serum concentrations of calcium, alkaline phosphatase, lipids, HbA1c and glucose were measured with routine laboratory methods.

Statistics

In the patients, the long-term development of BMD, body composition and laboratory parameters was analysed by means of the generalized estimating equation (GEE). GEE-analysis was used because repeated measurements within patients are dependent. In the GEE-analysis, time was treated as a categorical variable and therefore represented by dummy variables. This is in order not to assume a certain function over time.²¹ Because the BMD measurements in the control subjects were only performed at baseline and 10 years later, differences in development between patients and controls were analysed with an analysis of covariance; that is a linear regression analysis with the measurement at 10 years as outcome, correcting for the baseline value and with the group indicator (patient vs. control) as determinant. GEE-analysis were performed with STATA-version 7,²² the

analysis of covariance and *t*-tests were performed with SPSS-version 11. $P < 0.05$ is significant.

Results

Demographic data

With regard to age and BMI, there were no group differences in baseline and 10 years' follow-up data for the patients and control subjects. Mean age at baseline was 28.6 ± 4.2 years (range 20–40) for the patients and 29.7 ± 4.6 years (range 23–38) for the matched control subjects. Mean BMI in the patients at baseline was 21.9 ± 2.3 and in the control subjects 23.3 ± 1.3 . BMI 10 years later in the patients was 25.9 ± 3.2 and in the control subjects 25.1 ± 2.1 .

Changes in BMD in GH-deficient patients

BMD in the lumbar spine (L2–L4) increased significantly in the patients after 1 year of GH substitution and continued to increase during the 10 years of follow-up. With regard to the hip, BMD of the femoral neck and trochanter region increased (compared to baseline) after 1 year until 5 years in the GH-deficient patients, but between 5 and 10 years there was a decrease (Fig. 1). Values after 10 years were not different from baseline values.

Changes in BMD in GH-deficient patients compared to control subjects

Analysis of covariance showed that BMD in the lumbar spine in patients changed significantly compared to controls (difference 0.06 g/cm^2 , $P = 0.01$). BMD in the lumbar spine in the patients changed from $0.90 \pm 0.15 \text{ g/cm}^2$ at baseline to $0.96 \pm 0.12 \text{ g/cm}^2$ after 10 years, while in the control subjects lumbar BMD changed from 1.10 ± 0.14 to $1.05 \pm 0.11 \text{ g/cm}^2$. In addition, femoral neck BMD changed significantly compared to controls (difference 0.06 g/cm^2 , $P = 0.03$). Femoral neck BMD in the patients changed from $0.78 \pm 0.12 \text{ g/cm}^2$ at baseline to $0.80 \pm 0.10 \text{ g/cm}^2$ after 10 years. In the control subjects femoral neck BMD changed from 0.94 ± 0.13 to $0.83 \pm 0.10 \text{ g/cm}^2$ after 10 years. BMD measurements in the trochanter region did not change significantly in patients and control subjects over the 10 years of follow-up (difference 0.02 g/cm^2 , $P = 0.23$). When a correction was made for BMI, a smaller difference in BMD changes in the patients compared to the control subjects was found, which was no longer significant (lumbar spine difference 0.06 g/cm^2 , $P = 0.06$; femoral neck difference 0.07 g/cm^2 , $P = 0.05$).

Changes in body composition

BMI and leg and arm circumference increased significantly during the 10 years of GH substitution. Waist–hip ratio (WHR) decreased significantly compared to baseline after 1, 2, 3 and 4 years and was not different from baseline after 5 years. After 10 years of GH substitution WHR had significantly increased compared to baseline. Triceps skinfold thickness decreased significantly during the first 5 years of follow-up, but was not different compared to baseline after 10 years (see Table 1).

Table 1. Body composition at baseline and during follow-up (mean (95% confidence interval)) in the GH-deficient patients

	Baseline	1 year	2 years	3 years	4 years	5 years	10 years
BMI (kg/m ²)	21.9 (21.0–22.8)	22.7* (21.3–24.1)	23.4* (22.0–24.7)	24.3* (22.9–25.6)	24.4* (23.0–25.8)	24.6* (23.2–26.0)	25.9* (24.2–27.6)
WHR	0.92 (0.91–0.94)	0.89* (0.87–0.92)	0.90* (0.87–0.92)	0.90* (0.87–0.93)	0.91* (0.88–0.94)	0.93 (0.88–0.97)	0.97* (0.93–1.0)
LC (cm)	48.5 (47.1–50.1)	49.7* (47.1–52.4)	50.7* (48.0–53.4)	51.7* (49.2–54.3)	52.0* (49.4–54.7)	51.9* (49.2–54.7)	52.6* (49.5–55.7)
AC (cm)	27.5 (26.7–28.3)	27.8 (26.5–29.1)	28.6* (27.1–30.0)	29.3* (28.0–30.5)	30.0* (28.5–31.4)	30.2* (28.7–34.7)	30.7* (29.2–32.2)
Triceps SF (mm)	15.0 (13.2–16.8)	9.4* (6.2–12.6)	10.4* (7.0–13.9)	11.0* (7.3–14.8)	11.1* (7.9–14.3)	10.8* (7.4–14.3)	15.6 (10.9–20.2)

*Significantly different compared to baseline.

BMI, body mass index; WHR, waist–hip ratio; LC, leg circumference; AC, arm circumference; SF, skinfolds.

Table 2. Laboratory parameters at baseline and during follow-up in the GH-deficient patients (mean (95% confidence interval))

	Reference value	Baseline	1 year	2 years	3 years	4 years	5 years	10 years
Mean GH dose (mg/day)			0.97 (0.78–1.16)	1.07 (0.98–1.45)	0.83 (0.75–0.92)	0.71 (0.61–0.81)	0.58 (0.49–0.67)	0.40 (0.30–0.50)
IGF-I (nmol/l)	Age and sex dependent†	9.7 (7.6–11.8)	43.8* (35.6–52.0)	47.6* (39.0–56.2)	44.4* (35.3–53.5)	37.1* (29.2–45.0)	35.4* (29.5–41.4)	26.6* (20.5–32.7)
Osteocalcin (nmol/l)	0.4–4.2	2.8 (2.1–3.6)	6.5* (4.5–8.5)	7.8* (6.3–9.3)	7.4* (5.6–9.2)	5.1* (3.5–6.7)	na	2.5 (0.8–4.1)
Alkaline phosphatase (U/l)	< 90	58.0 (50.4–65.6)	115.2* (99.3–131.1)	83.0* (68.3–97.7)	69.7* (54.2–85.3)	60.7 (45.2–76.2)	52.4 (38.7–66.1)	50.5 (38.3–62.7)
Calcium (mmol/l)	2.20–2.60	2.31 (2.27–2.35)	2.40* (2.31–2.48)	2.36* (2.27–2.45)	2.35 (2.27–2.44)	2.31 (2.20–2.41)	2.30 (2.22–2.38)	2.37* (2.30–2.45)
Urine calcium/creatinine	< 0.45	0.13 (0.10–0.16)	0.24* (0.15–0.32)	0.21* (0.11–0.30)	0.19* (0.11–0.27)	na	0.46* (0.11–0.81)	0.25 (0.16–0.34)
Urine HOP/creatinine (mmol/mol creatinine)	< 25	18.1 (14.4–21.8)	66.8* (50.4–83.1)	43.3* (32.7–54.0)	34.3* (25.3–43.3)	na	28.6* (19.8–37.4)	20.8 (12.8–28.9)
HbA1c (%)	4.3–6.1	4.8 (4.6–5.0)	5.0* (4.6–5.4)	5.1* (4.7–5.4)	5.0* (4.7–5.4)	5.1* (4.7–5.5)	5.0* (4.6–5.4)	5.2* (4.1–5.2)
Glucose (mmol/l)	3.8–6.4	4.1* (3.9–4.3)	4.5* (4.2–4.8)	4.9* (4.5–5.2)	4.9* (4.5–5.4)	4.7* (4.1–5.3)	4.5* (4.0–5.0)	4.6* (4.1–5.2)
Total cholesterol (mmol/l)	< 6.5	6.1 (5.7–6.6)	5.3* (4.6–6.1)	5.4* (4.5–6.2)	5.1* (4.1–6.0)	5.1* (4.2–6.0)	4.9* (4.0–5.9)	5.7 (4.7–6.7)
LDL-cholesterol (mmol/l)	< 5.0	4.3 (3.9–4.8)	3.6* (2.8–4.3)	3.7* (2.9–4.5)	3.4* (2.6–4.3)	3.3* (2.5–4.3)	3.3* (2.3–4.3)	3.6* (2.9–4.4)
HDL-cholesterol (mmol/l)	> 0.9	1.1 (1.0–1.2)	1.1 (0.9–1.4)	1.1 (0.9–1.3)	1.1 (0.9–1.3)	1.1 (0.9–1.3)	1.3* (1.0–1.5)	1.5* (1.2–1.7)

*Significant difference compared to baseline ($P < 0.05$); na, not available; HOP, hydroxyproline.

†Reference values for IGF-I in men: age 25–36, 23 nmol/l (range 10–47); age 36–46, 17 nmol/l (range 10–30).

Changes in laboratory parameters

Table 2 shows laboratory parameters at baseline and during follow-up in the GH-deficient patients. Serum IGF-I levels were significantly increased compared to baseline during follow-up. Serum IGF-I levels after 4 years were significantly lower than after 3 years ($P = 0.03$), and values after 10 years were significantly lower than after 5 years

($P = 0.03$). Serum osteocalcin, as a marker of bone synthesis, increased significantly during the first 4 years of follow-up, but was not different from baseline after 10 years. Serum values of alkaline phosphatase increased significantly during the first 3 years of GH substitution, but were not significantly different from baseline after 4, 5 and 10 years. Serum calcium concentrations were significantly increased after 1, 2 and 10 years. Urine calcium/creatinine ratio

increased significantly during the first 5 years of follow-up, but was not significantly different from baseline after 10 years. Urine hydroxyproline/creatinine ratio increased significantly after 1, 2, 3 and 5 years, but values after 10 years were not significantly different from baseline.

Serum HbA1c and fasting serum glucose increased significantly during the 10-year follow-up period. Lipid profile improved during follow-up as total cholesterol decreased significantly after 1, 2, 3, 4 and 5 years. However, after 10 years total cholesterol was not significantly different from the baseline values. LDL-cholesterol decreased significantly during the whole follow-up period, while high density lipoprotein (HDL)-cholesterol increased significantly after 5 and 10 years of GH substitution. Triglycerides did not change significantly during follow-up in this patient group (data not shown).

Mean GH dose and concomitant medication

Table 2 shows the mean GH dose during follow-up in the patients. During the follow-up, the GH dose significantly decreased after 2 years. Seven patients used temporarily concomitant medication besides their hormonal substitution during the 10 years of follow-up. Indications for these medications were high cholesterol ($n = 5$), asthmatic bronchitis ($n = 2$) and depression ($n = 1$). Other concomitant medications used were antihistaminergic drugs ($n = 2$) and proton pump inhibitors ($n = 3$). None of the patients used bisphosphonates or other concomitant medication known to affect bone metabolism.

Baseline values in patients who continued and discontinued GH treatment

Baseline data for the 15 patients who were evaluated after 5 years but who did not continue GH substitution during the 10-year follow-up period were compared to data for the 23 patients who continued GH therapy for 10 years. The baseline BMD values for the hip were significantly higher in the 15 patients who discontinued GH therapy in the past 5 years compared to the patients who continued GH (femoral neck 0.89 ± 0.16 vs. 0.77 ± 0.12 ; $P = 0.02$, and trochanter 0.80 ± 0.14 vs. 0.70 ± 0.12 ; $P = 0.03$).

Discussion

In this 10-year follow-up study of GH substitution in GH-deficient adults we have shown that long-term GH replacement is beneficial to maintain improvements in BMD in the lumbar spine. BMD of the lumbar spine in the GH-deficient patients increased compared to baseline, while a decrease in BMD was shown in the lumbar spine in the control subjects during the 10 years of follow-up. BMD of the hip (femoral neck and trochanter) in the control subjects also decreased significantly compared to baseline. In the femoral neck of the control subjects we observed a decline of 12% in BMD, and BMD in the lumbar spine declined by 5% in the control subjects. The values for the spine are in accordance with another 10-year longitudinal study in healthy adult men.²³ The changes in the hip were not measured in that study. As there are no other studies that report the natural decline in BMD in the hip in healthy men aged 30–40 years, we

cannot compare this large decline. In our patients, BMD in the hip after 10 years was not significantly different from baseline values, but it was lower than after 5 years.

A 7-year follow-up study of long-term skeletal effects of GH substitution (GH alone or combined with a bisphosphonate, alendronate) in GH-deficient adults showed similar results, as we found in this study. There was a significant increase in lumbar spine BMD in the first 4 years of GH therapy. However, after 4 years of GH substitution, BMD seemed to reach a plateau, as no significant increase was found in the 3–4 years thereafter.²⁴ It seems that BMD stabilizes and values are maintained for up to 7 or 10 years. Valimaki *et al.* demonstrated no further increase in BMD (lumbar spine and hip) after more than 4 years of GH substitution in patients with GHD.²⁵ The pathophysiological basis for these observations is not known. In the control subjects, we observed a decrease in BMD in both the lumbar spine and the hip. It is known that peak bone mass is reached during young adulthood (around the age of 30 years), thereafter BMD decreases slowly with increasing age. We demonstrated this natural decrease during the 10 years of follow-up in our control subjects, but not in our patients.

Serum concentrations of bone markers were elevated compared to baseline during the first 5 years of the study, but were not different from baseline after 10 years. A possible explanation could be that the bone remodelling in GH-deficient patients after long-term GH substitution decreases due to lower GH substitution doses. Serum IGF-I levels slowly decreased during the 10 years of GH substitution, but levels were always elevated compared to baseline. The international recommended GH doses have decreased in recent years and the patients are 10 years older compared to baseline, implying that recommended doses (age- and sex-dependent) are lower at the end of the study.

BMD measurements were performed with two different DEXA instruments. This problem arises frequently in long-term follow-up studies, as the device used during the first 5 years of this study was replaced and so measurements after 10 years were made with another device. We tried to resolve this problem by measuring BMD in both the patients and the control subjects on both devices. In addition, we measured the ESP on both devices and results showed very similar BMD values. The Hologic-4500 measures a lower BMD compared to the Norland XR-26, so observed changes in BMD in our study from baseline to 10 years' follow-up can be an underestimation of the true BMD changes over time.

A limitation of this follow-up study is the lack of data on the patients who discontinued their GH substitution. For different reasons this group was unable to serve as a control group. Some of the patients who discontinued GH therapy were lost to follow-up and some others restarted GH substitution after some time without GH therapy. The heterogeneous group of patients who stopped their GH substitution made it impossible to compare results with patients who continued their GH substitution therapy. It could be argued that positive effects found in this study are partly due to selection bias. However, patients had different reasons for discontinuing their GH therapy (e.g. lack of efficacy, adverse events). Post-hoc analyses of baseline IGF-I values of all patients included at the start of the study ($n = 50$) revealed that baseline IGF-I values were similar in the patients ($n = 23$) who continued GH for 10 years (8.4 ± 4.9) and in

the patients who discontinued GH therapy ($n = 27$) during follow-up (10.7 ± 6.1) ($P = 0.15$).

Baseline data for the 15 patients who were evaluated after 5 years but did not continue GH substitution during the 10-year follow-up period were compared to data for the 23 patients who continued GH therapy during the 10 years of follow-up. Analyses showed no differences for age, IGF-I values, BMI and biochemical values between these groups (data not shown). However, the baseline BMD values for the hip were significantly higher in the 15 patients who discontinued GH therapy in the past 5 years compared to the patients who continued GH. This could possibly have influenced the decision to discontinue GH therapy. However, both groups of patients showed a positive effect of GH substitution on BMD during the first years of the study.

Post-hoc analyses of the different types of GHD (IGHD vs. MPHD) in the patients who continued GH therapy for 10 years showed that the patients with isolated GHD ($n = 6$) had significantly higher IGF-I values at baseline compared to the patients with MPHD (15.3 ± 3.9 vs. 7.7 ± 3.9 ; $P = 0.001$). Serum calcium values were also higher in the isolated GH-deficient patients than in MPH-deficient patients (2.38 ± 0.08 vs. 2.29 ± 0.09 ; $P = 0.04$) and the urine calcium/creatinine ratios at baseline were elevated in the IGH-deficient patients compared to the MPH-deficient patients (0.19 ± 0.09 vs. 0.11 ± 0.07 ; $P = 0.04$). Other values at baseline (age, BMI, WHR, BMD) were not different and the effect of GH on BMD was also not different in the IGH- and MPH-deficient patients (data not shown).

Repeated BMD measurements are frequently used to monitor the effects of therapeutic interventions. In a recent study, reproducibility in postmenopausal women on the Hologic-4500 was good.²⁶ It was stated that a BMD change of at least $\pm 0.05 \text{ g/cm}^2$ at L1–L4 should be considered significant with repeated measurements on the same DEXA device. In our study we measured on different DEXA devices, but found an increase of 0.06 g/cm^2 for the lumbar spine in the patients. This could be considered as a significant difference.

The observed changes in lipid profile in our study are comparable to the findings in the only other 10-year follow-up study.¹⁵ In that study, a reduction in LDL-cholesterol and an increase in HDL-cholesterol were observed, while fasting glucose, total cholesterol and triglycerides did not change. We also found a decrease in LDL-cholesterol and an increase in HDL-cholesterol after 10 years. Post-hoc analyses of the lipid data after excluding the five patients who used medication for high cholesterol during the follow-up period showed similar favourable changes of the lipid profile during 10 years as those for the whole group (data not shown).

Our results of an increase in fasting glucose levels are in line with previous findings in a 5-year follow-up study²⁷ in which serum glucose and insulin levels increased after 1 year. It was suggested that the reduction in percentage body fat after long-term treatment would overcome the negative effects of GH on glucose metabolism. However, we found an increase in glucose levels and no positive effect of GH on body weight after 10 years. Body composition in the other 10-year follow-up study in GH-deficient patients showed an increase in lean body mass, with a reduction in fat mass after 10 years.¹⁵ We found an increase in BMI and WHR. Interobserver variability can be an explanation for the increase in WHR as observations after 10 years were made by a different observer than during the first years

of the study. However, increased WHR is in line with the increased BMI, arm and leg circumferences. Also in line with our results in the healthy volunteers are the results of a 10-year longitudinal study of body composition and lumbar bone mass in 225 men and 241 women measured at age 27, 32 and 36 years.²³ In the men there was an increase of 10% of BMI and an increase of 5.5% in WHR. The same study showed a decrease of 5% in BMD of the lumbar spine in healthy men. We found an increase in BMD in the lumbar spine of our patients during the 10 years of follow-up, but the BMD in the hip increased during the first 5 years of follow-up, but not after 10 years. A possible explanation for this could be that the level of IGF-I needed to increase BMD is different for the hip and lumbar spine. After 2 years the mean GH dose and serum IGF-I levels decreased, and possibly the lumbar spine does not need a high IGF-I level to promote bone formation. Another explanation could be the interaction between increased body weight and increased BMD in the lumbar spine.

Sex steroid hormones and thyroid hormone are known to influence bone density in patients with hypopituitarism. The substitution with sex and thyroid hormone in our MPH-deficient patients was sufficient during the whole follow-up study, with regular laboratory checks and dose adjustments if necessary.

All patients were retested for GHD after reaching final height with a GHRH stimulation test or an ITT. An inclusion criterion was a maximum GH response $< 7 \mu\text{g/L}$. Nowadays an ITT is recommended to assess GHD in adults and cut-off values are $< 3 \mu\text{g/L}$. In our 23 patients there were 19 patients with a maximum GH response $< 3 \mu\text{g/L}$. The other four patients with a GH response between 3 and $7 \mu\text{g/L}$ were all deficient for multiple pituitary hormones and were therefore classified as GH deficient.

In conclusion, this 10-year follow-up study has shown that 10 years of GH replacement in adult patients with CO-GHD results in an improved lipid profile and increased BMD in the lumbar spine. After 10 years, there was no increase in BMD in the hip compared to baseline values, while BMD in healthy control subjects decreased during follow-up. Bone markers were elevated during the study, but after 10 years returned to values at baseline. BMI and WHR were increased after 10 years compared to baseline. Our findings suggest that long-term GH substitution is beneficial to preserve improvements in BMD.

References

- 1 Shimon, I. (2003) Growth hormone replacement for adult growth hormone deficiency. *Expert Opinion in Pharmacotherapy*, **4**, 1977–1983.
- 2 Cuneo, R.C., Salomon, F., McGauley, G.A. & Sonksen, P.H. (1992) The growth hormone deficiency syndrome in adults. *Clinical Endocrinology*, **37**, 387–397.
- 3 de Boer, H., Blok, G.J. & van der Veen, E.A. (1995) Clinical aspects of growth hormone deficiency in adults. *Endocrine Reviews*, **16**, 63–86.
- 4 Baum, H.B., Biller, B.M., Finkelstein, J.S., Cannistraro, K.B., Oppenheim, D.S., Schoenfeld, D.A., Michel, T.H., Wittink, H. & Klibanski, A. (1996) Effects of physiologic growth hormone therapy on bone density and body composition in patients with adult-onset growth hormone deficiency. A randomized, placebo-controlled trial. *Annals of Internal Medicine*, **125**, 883–890.

- 5 Johannsson, G., Rosen, T., Bosaeus, I., Sjostrom, L. & Bengtsson, B.A. (1996) Two years of growth hormone (GH) treatment increases bone mineral content and density in hypopituitary patients with adult-onset GH deficiency. *Journal of Clinical Endocrinology and Metabolism*, **81**, 2865–2873.
- 6 Deijen, J.B., de Boer, H. & van der Veen, E.A. (1998) Cognitive changes during growth hormone replacement in adult men. *Psychoneuroendocrinology*, **23**, 45–55.
- 7 Drake, W.M., Howell, S.J., Monson, J.P. & Shalet, S.M. (2001) Optimizing GH therapy in adults and children. *Endocrine Reviews*, **22**, 425–450.
- 8 O'Halloran, D.J., Tsatsoulis, A., Whitehouse, R.W., Holmes, S.J., Adams, J.E. & Shalet, S.M. (1993) Increased bone density after recombinant human growth hormone (GH) therapy in adults with isolated GH deficiency. *Journal of Clinical Endocrinology and Metabolism*, **76**, 1344–1348.
- 9 Holmes, S.J., Economou, G., Whitehouse, R.W., Adams, J.E. & Shalet, S.M. (1994) Reduced bone mineral density in patients with adult onset growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism*, **78**, 669–674.
- 10 de Boer, H., Blok, G.J., van Lingen, A., Teule, G.J., Lips, P. & van der Veen, E.A. (1994) Consequences of childhood-onset growth hormone deficiency for adult bone mass. *Journal of Bone and Mineral Research*, **9**, 1319–1326.
- 11 Kaufman, J.M., Taelman, P., Vermeulen, A. & Vandeweghe, M. (1992) Bone mineral status in growth hormone-deficient males with isolated and multiple pituitary deficiencies of childhood onset. *Journal of Clinical Endocrinology and Metabolism*, **74**, 118–123.
- 12 Davidson, P., Milne, R., Chase, D. & Cooper, C. (2004) Growth hormone replacement in adults and bone mineral density: a systematic review and meta-analysis. *Clinical Endocrinology*, **60**, 92–98.
- 13 van der Sluis, I., Boot, A.M., Hop, W.C., De Rijke, Y.B., Krenning, E.P. & de Muinck Keizer-Schrama, S.M. (2002) Long-term effects of growth hormone therapy on bone mineral density, body composition, and serum lipid levels in growth hormone deficient children: a 6-year follow-up study. *Hormone Research*, **58**, 207–214.
- 14 Maison, P., Griffin, S., Nicoue-Beglah, M., Haddad, N., Balkau, B. & Chanson, P. (2004) Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a meta-analysis of blinded, randomized, placebo-controlled trials. *Journal of Clinical Endocrinology and Metabolism*, **89**, 2192–2199.
- 15 Gibney, J., Wallace, J.D., Spinks, T., Schnorr, L., Ranicar, A., Cuneo, R.C., Lockhart, S., Burnand, K.G., Salomon, F., Sonksen, P.H. & Russell-Jones, D. (1999) The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. *Journal of Clinical Endocrinology and Metabolism*, **84**, 2596–2602.
- 16 ter Maaten, J.C., de Boer, H., Kamp, O., Stuurman, L. & van der Veen, E.A. (1999) Long-term effects of growth hormone (GH) replacement in men with childhood-onset GH deficiency. *Journal of Clinical Endocrinology and Metabolism*, **84**, 2373–2380.
- 17 de Boer, H., Blok, G.J., Voerman, B., Derriks, P. & van der Veen, E.A. (1996) Changes in subcutaneous and visceral fat mass during growth hormone replacement therapy in adult men. *International Journal of Obesity*, **20**, 580–587.
- 18 Ooms, M.E., Roos, J.C., Bezemer, P.D., van der Vijgh, W.J., Bouter, L.M. & Lips, P. (1995) Prevention of bone loss by vitamin D supplementation in elderly women: a randomized double-blind trial. *Journal of Clinical Endocrinology and Metabolism*, **80**, 1052–1058.
- 19 de Boer, H., Blok, G.J., Voerman, H.J., De Vries, P.M. & van der Veen, E.A. (1992) Body composition in adult growth hormone-deficient men, assessed by anthropometry and bioimpedance analysis. *Journal of Clinical Endocrinology and Metabolism*, **75**, 833–837.
- 20 Teerlink, T., Tavenier, P. & Netelenbos, J.C. (1989) Selective determination of hydroxyproline in urine by high-performance liquid chromatography using precolumn derivatization. *Clinica et Chimica Acta*, **183**, 309–315.
- 21 Twisk, J.W. (2003) The problem of evaluating the magnitude of tracking coefficients. *European Journal of Epidemiology*, **18**, 1025–1026.
- 22 Twisk, J.W. (2004) Longitudinal data analysis. A comparison between generalized estimating equations and random coefficient analysis. *European Journal of Epidemiology*, **19**, 769–776.
- 23 Bakker, I., Twisk, J.W., van Mechelen, W. & Kemper, H.C. (2003) Fat-free body mass is the most important body composition determinant of 10-year longitudinal development of lumbar bone in adult men and women. *Journal of Clinical Endocrinology and Metabolism*, **88**, 2607–2613.
- 24 Biermasz, N.R., Hamdy, N.A., Pereira, A.M., Romijn, J.A. & Roelfsema, F. (2004) Long-term skeletal effects of recombinant human growth hormone (rhGH) alone and rhGH combined with alendronate in GH-deficient adults: a seven-year follow-up study. *Clinical Endocrinology*, **60**, 568–575.
- 25 Valimaki, M.J., Salmela, P.I., Salmi, J., Viikari, J., Kataja, M., Turunen, H. & Soppi, E. (1999) Effects of 42 months of GH treatment on bone mineral density and bone turnover in GH-deficient adults. *European Journal of Endocrinology*, **140**, 545–554.
- 26 Lodder, M.C., Lems, W.F., Ader, H.J., Marthinsen, A.E., van Coeverden, S.C., Lips, P., Netelenbos, J.C., Dijkman, B.A. & Roos, J.C. (2004) Reproducibility of bone mineral density measurement in daily practice. *Annals of Rheumatic Diseases*, **63**, 285–289.
- 27 Giavoli, C., Porretti, S., Ronchi, C.L., Cappiello, V., Ferrante, E., Orsi, E., Arosio, M. & Beck-Peccoz, P. (2004) Long-term monitoring of insulin sensitivity in growth hormone-deficient adults on substitutive recombinant human growth hormone therapy. *Metabolism*, **53**, 740–743.