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Growth Hormone Replacement Therapy Improves Body Composition and Increases Bone Metabolism in Elderly Patients with Pituitary Disease*

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ABSTRACT

Although a specific GH deficiency (GHD) syndrome in the adult and the response to GH replacement therapy are well recognized, there are few data available on the effect of GH replacement therapy in elderly GH-deficient patients. We studied the effect of GH therapy on body composition and bone mineral density measured by dual energy x-ray absorptiometry, markers for bone metabolism, insulin-like growth factors (IGFs), and IGF-binding proteins (IGFBPs) in 31 patients (6 women and 25 men; aged 60–79 yr; mean, 68 yr) with multiple pituitary hormone deficiencies. The GH response to arginine or insulin was below 3 μg/L (9 mU/L) in all subjects. They were randomized to GH (Humatrope, Eli Lilly & Co.) or placebo for 6 months, followed by 12 months of open treatment. The dose was 0.05 IU/kg·week for 1 month, and after that it was 0.1 IU/kg·week divided into daily sc injections (0.75–1.25 IU/day).

There were no changes in any of the measured variables during placebo treatment. GH treatment normalized serum IGF-I in a majority of the patients and increased IGFBP-3 and -5 as well as IGFBP-4 and IGF-II to values within normal range. Lean body mass was increased, and the increase at 6 and 12 months correlated with the increase in IGF-I ($r = 0.46$; $P = 0.010$ and $r = 0.54$, respectively; $P = 0.003$). GH treatment caused a modest, but highly significant, reduction of total body fat. Mean bone mineral density was not different from that in healthy subjects of the same age and did not change during the observation period. Markers for bone formation (bone-specific alkaline phosphatase activity, osteocalcin, and procollagen I carboxyl-terminal peptide in serum) increased within the normal range, and levels were sustained throughout the study. The bone resorption marker (pyridinoline in urine) was significantly elevated for 12 months. Side-effects were mild, mostly attributed to fluid retention. In two patients with normal glucose tolerance at the start of the study, pathological glucose tolerance occurred in one patient and was impaired in one.

In conclusion, elderly patients with GHD respond to replacement therapy in a similar manner as younger subjects, with an improvement in body composition and an increase in markers for bone metabolism. Side-effects are few, and elderly GHD patients can be offered treatment. As long-term risks are unknown, GH doses should be titrated to keep IGF-I within the age-related physiological range. (*J Clin Endocrinol Metab* **85:** 4104–4112, 2000)

THE IMPORTANCE OF adequate GH secretion during adult life is now well recognized. A specific adult GH deficiency (GHD) syndrome, has been described and recently reviewed (1). These patients have abnormal body composition with decreased muscle mass and increased adipose tissue mass (1). In addition, they have low bone mineral density (BMD), in particular with childhood-onset GHD (1–4). In patients with adult-onset GHD, both low (5) and normal BMD values (6) have been reported. With GH replacement therapy, changes in body composition can be reversed (1, 7–10); bone turnover, as indicated by markers of bone metabolism, and subsequently BMD increase (1, 3, 11).

Despite the numerous publications on the effects of GH replacement therapy in adults in recent years, there is a paucity of information on the characteristics and the response to replacement in elderly GHD patients. The GH production rate declines by age and from 40 yr of age decreases about 14%/decade, primarily due to a decrease in the amplitude of GH pulses (12, 13). Despite the low GH secretion in old age, there was a significant reduction of spontaneous GH secretion and the GH response to arginine when patients with pituitary disease were compared with age-matched healthy elderly subjects (14). This indicates that older patients with GHD may also benefit from GH therapy.

Previous studies in younger adults have shown a wide interindividual variation in the GH-induced changes in body composition and BMD, and there is a need for serum markers that can predict these effects. As many of anabolic GH effects are mediated via stimulation of insulin-like growth factor I (IGF-I), circulating IGF-I levels could be a putative marker. However, we have found only weak correlations between the levels of or changes in lean body mass (LBM) and IGF-I levels during GH replacement therapy (10). Moreover, at least in the short term (1 week), a low GH dose (2 μg/kg·day) was sufficient to normalize serum IGF-I without affecting whole body protein and lipid kinetics (15).

The majority of IGFs exists as complexes bound to the six known fully characterized IGF-binding proteins (IGFBPs),

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which modulate IGF actions in both a positive and a negative manner (16, 17). IGFBP-1, IGFBP-2, and IGFBP-4 have been found to inhibit IGF actions, whereas the GH-dependent binding proteins, IGFBP-3 and IGFBP-5, can enhance the effects of IGF. IGF-independent effects of IGFBPs have also been described (18, 19). BMD has been found to have a positive relationship to serum levels of IGFBP-5 during GH replacement therapy (20). The potential efficacy of IGFBP-5 and -4 as markers for the changes in LBM has not been evaluated.

The aim of the present work was to study baseline characteristics and the effects of GH replacement therapy on body composition, markers for bone metabolism, and BMD as well as on levels of IGFs and IGFBP-1 through -5 in patients with adult-onset GHD, aged 60–79 yr. In addition we wanted to evaluate whether the IGFs and IGFBP-1 through -5 could serve as markers for potential changes in body composition and BMD.

Subjects and Methods

Patients and diagnosis

Thirty-one patients, aged 60–79 yr (6 women and 25 men), with adult-onset pituitary disease with a known duration of 0.5–40 yr (mean, 11 yr) participated (Table 1). They were treated at 3 centers in Sweden; Department of Endocrinology and Diabetology, Karolinska Hospital, Stockholm; Department of Endocrinology, University Hospital Malmo, Malmo; and Department of Medicine, Umea University Hospital, Umea. Written informed consent was obtained from each patient, and the study was approved by the regional ethics committees and the Swedish Medical Product Agency.

The majority had panhypopituitarism due to a pituitary tumor and its treatment. The diagnosis of GHD was based on an insufficient response to a standard GH provocation test with arginine (n = 29) or insulin (n = 2). All patients fulfilled the consensus criterion for severe GHD, *i.e.* a maximal peak GH response less than 3.0 μg/L (21). The mean maximal GH concentration was 0.328 ± 0.10 μg/L. All patients had serum IGF-I levels below mean for 70-yr-old healthy subjects. The patients received adequate and stable replacement therapy for ACTH and TSH deficiencies and had not received GH replacement previously. All males with LH/FSH deficiency had testosterone replacement, and one of the women had systemic treatment with conjugated estrogens. The patients had normal fasting blood glucose and no history of diabetes mellitus. One patient had evidence of impaired arterial circulation in the legs with intermittent claudication. Apart from their pituitary insufficiency the patients suffered from no other serious illness and were living independently. Four had well controlled benign hypertension.

Study design and protocol

During the first 6 months of the study the patients were randomized, in a double blinded and parallel fashion, to inject either biosynthetic human GH (Humatrope, Eli Lilly & Co., Stockholm, Sweden) or placebo as a single sc injection once daily at bedtime. The study was then continued unblinded for another 12 months with GH treatment to the whole group. The starting dose was 0.05 IU/kg·week the first month of treatment and then increased to 0.1 IU/kg·week for another 5 months. Thereafter, *i.e.* 6 months after the start of the study, all patients were again treated with 0.05 IU/kg·week for 4 weeks to avoid breaking the blind protocol and then with 0.1 IU/kg·week for 11 months. The total daily GH dose ranged from 0.75–1.25 IU (mean, 0.92 ± 0.2). The doses of T_4, cortisone acetate, and gonadal steroids were kept constant during the study.

The study was performed on an out-patient basis, and blood samples were drawn in the morning after an overnight fast. At the start of the study and after 1, 2, 3, 6, 7, 8, 9, 12, 15, and 18 months, a physical examination, including height, weight, blood pressure, heart rate, and side-effects were registered, and blood samples were collected for measurement of IGF-I, IGF-II, and IGFBP-1, -2, -3, -4, and -5 as well as routine chemistry tests. Markers for bone metabolism in serum and urine were analyzed in samples obtained before and after 3, 6, 9, 12, 15, and 18 months of treatment.

LBM, total body fat (TBF), and BMD of total body, lumbar spine, and proximal femur (femoral neck and trochanter region) were assessed before and after 6, 12, and 18 months of treatment. In 26 patients bone quality was also assessed by ultrasound of the calcaneus at the same points in time.

An oral glucose tolerance test (OGTT) was performed before the start of treatment and then at 6-month intervals. Blood glucose was assessed 2 h after ingestion of 75 g glucose. A 2-h blood glucose value below 7.8 mmol/L was considered as normal, 7.8–11.1 mmol/L was defined as impaired glucose tolerance, and values above 11.1 mmol/L were considered pathological.

Assays

IGF-I was determined in serum by RIA after separation of IGFs from IGFBPs by acid-ethanol extraction and cryoprecipitation and with des(1–3)IGF-I as the radioligand (22) to minimize interference of remaining IGFBPs in the extract. The detection limit was 8 μg/L. Including the extraction step, the intra- and interassay coefficients of variation (CVs) were 4% and 11%, respectively. The normal range of IGF-I, which declines by age, was established in 448 healthy subjects, aged 20–96 yr (23). The geometrical mean concentration was 227 μg/L at 20 yr of age (range, 159–481 μg/L), 135 μg/L at 65 yr of age (78–235 μg/L), and 115 μg/L at 75 yr of age (range, 66–200 μg/L). The IGF-I values were also expressed as SD scores calculated from the regression line of values in these subjects.

Levels of IGF-II in serum were determined by a specific RIA as described by Blum and co-workers (24).

IGFBP-1 was analyzed by the method described by Povoa and coworkers (25). The sensitivity of the RIA was 3 μg/L, and the intra- and interassay CVs were 3% and 10%, respectively. The reference range was 7–100 μg/L.

IGFBP-2 was measured by the RIA described by Blum and co-workers (26).

IGFBP-3 was measured by RIA using a commercially available RIA kit with a slight modification (DSL 6700, Diagostics Systems Laborato-

TABLE 1. Patient characteristics in 31 elderly patients with GHD

	Total	GH treated	Placebo
No. of subjects	31	15	16
Males/females	25/6	14/1	11/5
Age, yr [mean (range)]	68 (60–79)	68 (60–77)	69 (19–79)
BMI, kg/m^2 [mean range]	25.5 (19.3–29.7)	25.3 (21.2–27.9)	25.7 (19.3–27.9)
IGF-I SD score [mean (range)]	−3.01 [−8.07– (−0.02)]	−3.27 [−6.41– (−0.82)]	−2.77 [−8.07– (−0.02)]
Panhypopituitarism (no.)	23	12	11
Pituitary adenoma (no.)	25	13	12
Idiopathic (no.)	2	1	1
Pituitary cyst (no.)	1	0	1
Empty sella syndrome (no.)	1	0	1
Post encephalitis (no.)	1	1	0
Craniopharyngioma (no.)	1	0	1

ries, Inc., Webster, TX). The intra- and interassay CVs were described as 5% and 7%, respectively. Cross-reactivity with IGFBP-1, -2 and -4 was less than 0.3%. The mean ± SD was 2966 ± 439 μg/L in individuals 50–70 yr of age.

IGFBP-4 in serum was measured by a RIA described by Honda and co-workers (27). Purified recombinant IGFBP-4 expressed in *Escherichia coli* resin was used as antigen, tracer, and standard. Intra- and interassay variations were less than 5% and 8%, respectively. There was no cross-reactivity with IGFBP-1, -2, -3, -5, or -6. The mean ± SD serum IGFBP-4 values in healthy men and women in the age group 61–87 yr was 546 ± 135 μg/L.

IGFBP-5 in serum was measured by a specific RIA described by Mohan and co-workers, using recombinant human IGFBP-5 as antigen, tracer, and standard (28). Intra- and interassay CVs were less than 4% and 8%, respectively. In 30 healthy women, aged 23–85 yr, the mean ± SD was 417 ± 194 μg/L.

Insulin in serum was measured by a conventional RIA technique.

Osteocalcin was determined by a commercial RIA kit (Cis-Bio osteocalcin ELSA), from CIS (Gif-sur-Yvette Cedex, France), with normal values for men of less than 30 μg/L and for postmenopausal women of less than 50 μg/L. Carboxyl-terminal propeptide of type I procollagen (PICP) was determined by RIA using a RIA kit from Orion Diagnostica with reference intervals (mean ± 2 SD) of 40–200 μg/L for men and 50–170 μg/L for women. Bone-specific alkaline phosphatase (ALP) activity was assessed by an immunoradiometric assay (Tandem-R Ostase, Hybritech, Fullerton, CA), with a normal value (mean ± SD) for men of 12.3 ± 4.3 μg/L and for women of 11.5 ± 4.3 μg/L. Urinary pyridinoline was determined by high pressure liquid chromatography (29, 30) in morning urine samples.

Body composition

LBM and TBF were determined by dual energy x-ray absorptiometry (DXA; DPX-L, Lunar Corp., Madison, WI) according to a standard procedure described previously (31). The same type of calibration phantom (Lunar Corp.) was used at all participating centers. The coefficient of variation was 1.6% for LBM and 4.0% for TBF.

BMD

BMD was measured by DXA (DPX-L, Lunar Corp.) of the total body, the lumbar spine (L2–L4), and the proximal femur (femoral neck and trochanter region). The measurements were made according to the standard procedure previously described (31). The precision of the method was 0.4% for total body BMD, 0.5% for spine BMD (L2–L4), and 1.6% for hip BMD (femoral neck). The BMD values in the patients were compared with data from reference material provided by the manufacturers. The BMD values were expressed as areal BMD (grams per cm^2) or as SD scores from the mean of age-matched reference material and SD scores from the mean of young adults (T scores).

Ultrasound measurements

Ultrasound measurements were made at the right and left os calcaneus using the Lunar Corp. Achilles ultrasound bone densitometer (32). The subject's heel was placed in a water bath at 37 C between two ultrasonic transducers. An acoustic wave with broad frequency band (100–600 kHz; center frequency, 500 kHz) was applied. Broad band ultrasound attenuation in decibels per MHz and speed of sound (SOS) in meter per second were calculated from the characteristics of the received signals. Broad band ultrasound attenuation and SOS were combined by the analysis software into a composite stiffness index, which was expressed as a percentage of age-matched and of young normal values. Calculations were based on the mean of the right and left heels.

Statistics

Results are presented as the mean ± SEM if not otherwise stated. To determine treatment effects of GH compared with baseline, normally and nonnormally distributed data were analyzed by one-way repeated measures ANOVA or Friedman's repeated measures ANOVA on ranks, respectively, both followed by Dunnett's test. The results from the first 6-month placebo-controlled period are presented separately; the differences between baseline values and the values at 6 months within the group were assessed by paired *t* test. In the comparison between the GH group and the placebo group regarding changes in parameters, the unpaired *t* test or Mann-Whitney rank sum test was used. Correlations between normally distributed variables were assessed using least square linear regression analysis. Relationships between variables with nonnormal distribution were analyzed by Spearman rank order correlation test. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SigmaStat for Windows (Jandel Scientific GmbH, Erkrath, Germany).

Results

As a result of the randomization procedure, 15 patients (1 woman and 14 men) constituted the group that received 18 months of active GH treatment, and 16 patients (5 women and 11 men) constituted the group that received 6 months of placebo followed by 12 months of active GH treatment (Table 1). At inclusion there was no difference between the groups in age (mean, 67.8 and 68.6 yr) and body mass index (25.3 and 25.7 kg/m^2). There was no difference between the groups regarding the etiology of pituitary insufficiency with 13 patients with pituitary adenomas in the GH group and 12 in the placebo group. Panhypopituitarism was found in 12 and 11 patients, respectively. Twenty-eight patients completed the whole study period. One patient who had history of intermittent claudication stopped treatment after 10 months due to vascular surgery of arterial insufficiency in a leg that subsequently led to an amputation. One patient withdrew without stating the reason after 15 months, and 1 patient did not take her GH injections the last month; all 3 of these patients received placebo the first 6 months and had active treatment for 4, 9, and 11 months, respectively. Thus, for data analysis, all patients were included during first 6-month placebo controlled period (15 patients receiving GH treatment and 16 patients receiving placebo). The effect of GH was then evaluated in the combined group of 28 patients receiving GH treatment for at least 12 months, and 15 patients comprised the group with active GH treatment for 18 months. In the placebo group, the visit at 6 months was considered comparable to the starting visit in the GH group.

Serum IGF, IGFBP, and insulin before and during treatment

At baseline, all patients had IGF-I levels below the normal mean for age, and two thirds had IGF-I levels below −2 SD (Fig. 1). IGF-II was below the normal mean in 16 individuals and 5 of them had values below −2 SD. Of the GH-dependent IGFBPs, IGFBP-3 concentrations were below −2 SD in 26, and IGFBP-5 levels were subnormal in 11 of the of the 31 patients. In addition, 16 of the patients had subnormal IGFBP-4 levels, whereas IGFBP-1, IGFBP-2, and insulin levels were within normal range. The levels of IGFBP-1 and insulin were negatively correlated ($r = -0.51$; $P < 0.01$), whereas the expected positive correlations were found between the GH-dependent peptides, *i.e.* IGF-I and IGFBP-3 ($r = 0.84$; $P < 0.001$), IGF-I and IGFBP-5 ($r = 0.61$; $P < 0.001$), and IGFBP-5 and IGFBP-3 ($r = 0.62$; $P < 0.001$). In addition, there was a positive correlation between IGFBP-4 and IGFBP-5 ($r = 0.53$; $P < 0.01$).

During the placebo-controlled first 6 months of the study there was no change in the mean levels of IGF-I, IGF-II, and

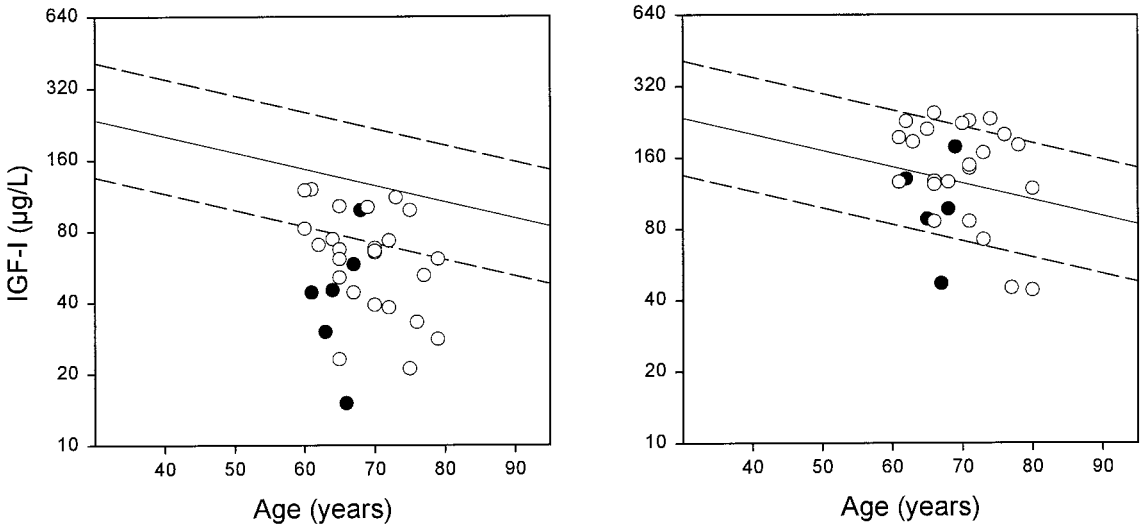

FIG. 1. Individual IGF-I concentrations at baseline (*left panel*; n = 31) and after 12 months replacement therapy (*right panel*; n = 28). ○, Male patients; ●, female patients. The normal range for age (the geometrical mean ± 2 SD) is indicated.

TABLE 2. Levels (mean ± SEM) of IGF-I, IGF-II, IGFBPs, insulin, and markers for bone before and after 6 months of GH or placebo therapy in patients aged 60–79 yr with adult-onset GHD

Peptide	GH (n = 15)			Placebo (n = 16)			
	0 months	6 months	P^a	0 months	6 months	P^a	P^b
IGF-I (μg/L)	55.8 ± 5.2	145 ± 14	<0.001	70.4 ± 9.0	70.0 ± 8.9	NS	<0.001
IGF-II (μg/L)	467 ± 36	555 ± 43	0.005	519 ± 49	456 ± 38	NS	0.013
IGFBP-3 (μg/L)	1218 ± 103	1914 ± 156	<0.001	1607 ± 184	1562 ± 174	NS	<0.001
IGFBP-5 (μg/L)	246 ± 21	315 ± 15	0.008	268 ± 19	291 ± 26	NS	0.043
IGFBP-4 (μg/L)	405 ± 29	455 ± 29	0.012	416 ± 32	439 ± 28	NS	0.012
IGFBP-2 (μg/L)	587 ± 82	476 ± 54	0.028	544 ± 81	570 ± 83	NS	0.013
IGFBP-1 (μg/L)	44.2 ± 9.2	31.5 ± 8.2	0.019	33.6 ± 6.2	33.1 ± 6.4	NS	0.043
Insulin (pmol/L)	48.8 ± 6.39	69.6 ± 9.3	0.026	63.1 ± 7.9	59.6 ± 8.6	NS	0.026
Osteocalcin (μg/L)	18.0 ± 1.3	32.2 ± 2.1	<0.001	20.5 ± 2.0	20.0 ± 1.7	NS	<0.001
PICP (μG/L)	76.0 ± 6.4	118 ± 9.6	<0.001	80.9 ± 6.6	79.8 ± 6.8	NS	0.001
ALP (μg/L)	8.8 ± 0.7	11.6 ± 1.1	<0.001	10.0 ± 1.2	9.3 ± 1.2	NS	<0.001
U-Pyridinoline (μg/L)	422 ± 42	633 ± 98	0.011	473 ± 101	384 ± 56	NS	0.010

[a] Comparison between baseline and 6 months within groups.
[c] Comparison of the change from 0 to 6 months during GH or placebo administration, respectively.

IGFBP-1 through -5 in the placebo group. In the GH-treated group, there was a significant rise in IGF-I, IGF-II, IGFBP-3, IGFBP-4, and IGFBP-5 and a decrease in IGFBP-1 and IGFBP-2 (Table 2). With the two groups combined, IGF-I increased during 12 months of treatment from a mean of 65.1 ± 5.5 μg/L to levels normal for age in the majority of the patients (mean, 145 ± 12 μg/L). Subnormal IGF-I levels at 12 months were found in three patients, of whom one was female (Fig. 1). The increase in IGF-I concentrations was sustained throughout the study (Fig. 2). IGF-II increased 21 ± 3.4% to values normal for age at 12 months and did not change thereafter. The mean levels were 489 ± 27, 598 ± 31, and 571 ± 39 μ/L at baseline, 12 months, and 18 months, respectively. IGFBP-3 had increased by 60 ± 7.4% after 12 months of treatment and did not change thereafter (Fig. 2). The mean molar ratio of IGF-I/IGFBP-3 increased from 0.269 at baseline to 0.389 at 12 months and 0.415 at 18 months ($P <$ 0.001). IGFBP-5, increased 30 ± 7.4% over 12 months, and the levels were similar at 18 months (Fig. 2). IGFBP-4 increased 9.3 ± 5.0% over 12 months and was unaltered thereafter (Fig. 2). At the start of treatment IGFBP-1 was 36.4 ± 5.5 μg/L, and IGFBP-2 was 525 ± 47 μg/L. Both were roughly within the normal range, and neither changed significantly seen over the 12/18-month period (Fig. 2). The fasting insulin level at baseline was 55.2 ± 5.7 pmol/L and rose to 68.9 ± 6.5 pmol/L after 6 months of GH treatment. No further rise was observed. The changes from baseline in IGF-I; IGF-II; IGFBP-3, -5, and −4; and insulin were significant at all times.

LBM and body fat before and during therapy and relationships to IGFs and binding proteins

During the first 6 months of the study there was no change in LBM in the placebo group; the mean values were 46.6 ± 2.7 and 46.9 ± 2.8 kg at baseline and 6 months, respectively. In the GH-treated group, LBM increased from 52.1 ± 1.6 to 54.5 ± 2.1 kg; the increase was significant ($P < 0.05$) compared to the change in the placebo group.

In the combined group (n = 28) significant elevations in LBM were found at all time points. At 6 months the mean increase was 1.9 ± 0.4 kg, at 12 months it was 1.8 ± 0.4 kg, and at 18 months it was 2.0 ± 0.6 kg, all compared with

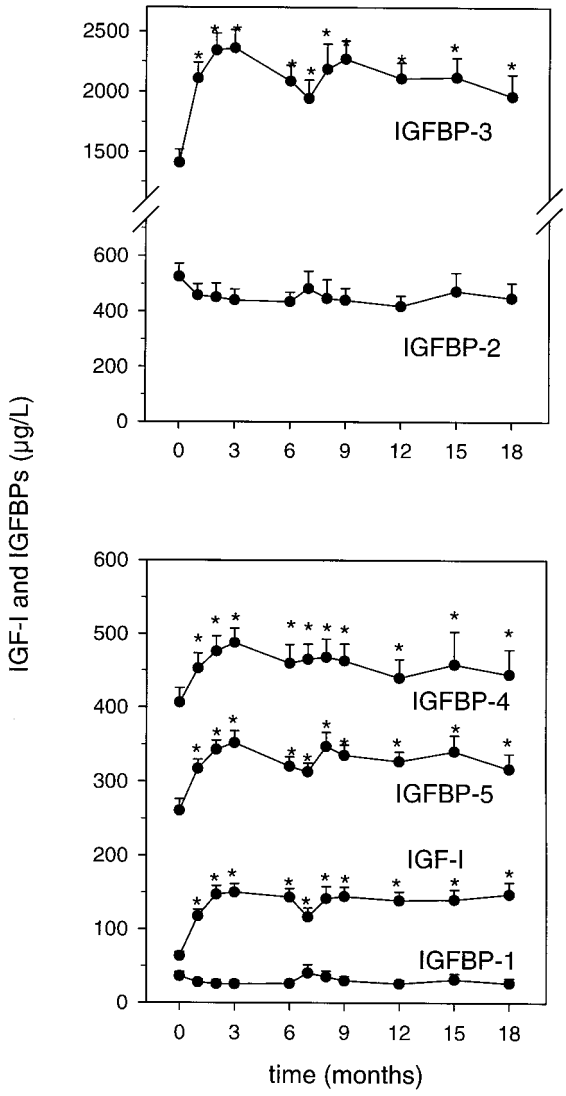

FIG. 2. Concentrations (mean ± SEM) of IGF-I and IGFBP-1, -2, -3, -4, and -5 before and during GH replacement therapy (n = 28 for 0–12 months; n = 15 for 15 and 18 months) in GHD patients, aged 60–79 yr. *, $P < 0.05$.

baseline. However, when an increase in LBM of 0.5 kg was arbitrarily defined as a significant response with consideration taken to practical clinical purposes and reproducibility, 8 of the 28 patients did not respond with an increase in LBM after 12 months of treatment. IGF-I levels at the start of the study were lower in the nonresponding group (51 ± 11 *vs.* 70 ± 6 μg/L) and did not increase as much as in the responding group over 12 months (32 ± 8 *vs.* 100 ± 9 μg/L). LBM changes were independent of the age of the patient, and responders and nonresponders had similar ages (68 ± 1.2 *vs.* 69 ± 2.2 yr). When the results from males and females were analyzed separately, it was found that mean LBM did not change in the females. Only 2 of the 5 females responded with an increase in LBM after 12 months (0.8 and 1.1 kg, respectively), whereas 18 of the 23 males during the same time showed an increase, ranging from 0.5–6.3 kg. The mean LBM increase in the males were 2.3 ± 0.5, 2.2 ± 0.4, and 2.1 ± 0.6 kg at 6, 12, and 18 months, respectively (Fig. 3).

LBM and IGF-I values correlated positively at 6 and 12 months (including both males and females; n = 28) with r = 0.40; $P < 0.05$ at 6 months and r = 0.55; $P < 0.01$ at 12 months. The increase in LBM from baseline at 6 and 12 months correlated with the increase in serum IGF-I (Fig. 4). LBM and its changes did not correlate to any of the IGFBPs.

TBF did not change during the 6-month placebo period; the mean values were 24.1 ± 1.1 and 24.1 ± 1.2 kg at baseline and 6 months, respectively. In the GH-treated group, mean TBF decreased from 22.3 ± 1.8 to 20.7 ± 1.6 kg, a significant decrease ($P < 0.05$) compared to the placebo group. In the combined group a significant decrease was found at 6, 12, and 18 months, with a decrease from 23.4 ± 1.1 to 21.7 ± 1.1 ($P < 0.05$) over 12 months. However, the mean values in the female patients were unchanged, whereas the decrease in the males was significant from 22.8 ± 1.3 to 21.1 ± 1.3 kg ($P < 0.001$; Fig. 3). TBF and its changes had no relationship to serum IGFs and binding proteins. Body mass index and waist/hip ratio remained unchanged.

BMD and markers for bone metabolism

At baseline, mean BMD measured with DXA was not different from that found in healthy age- and sex-matched

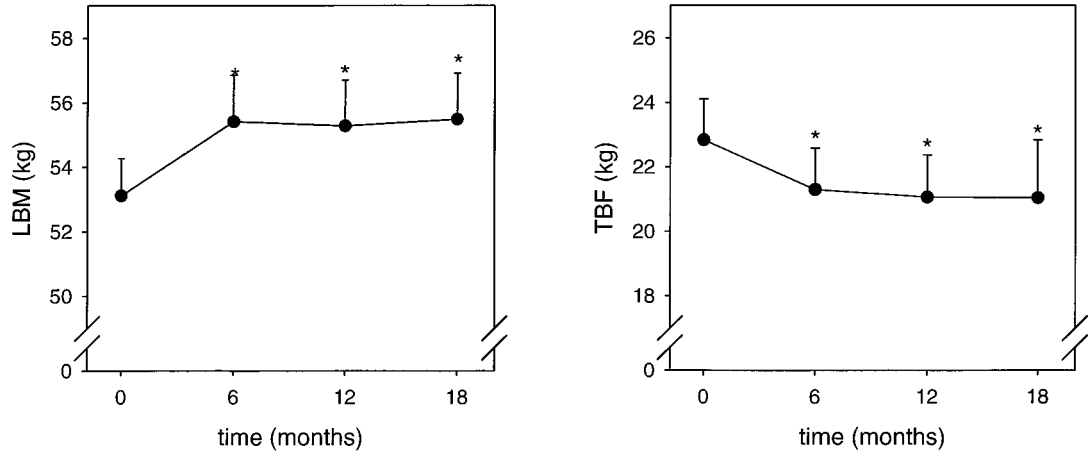

FIG. 3. LBM and TBF (mean ± SEM) before (n = 23) and after 6 (n = 23), 12 (n = 23), and 18 (n = 14) months in males, aged 60–79 yr, with GHD. *, $P < 0.05$.

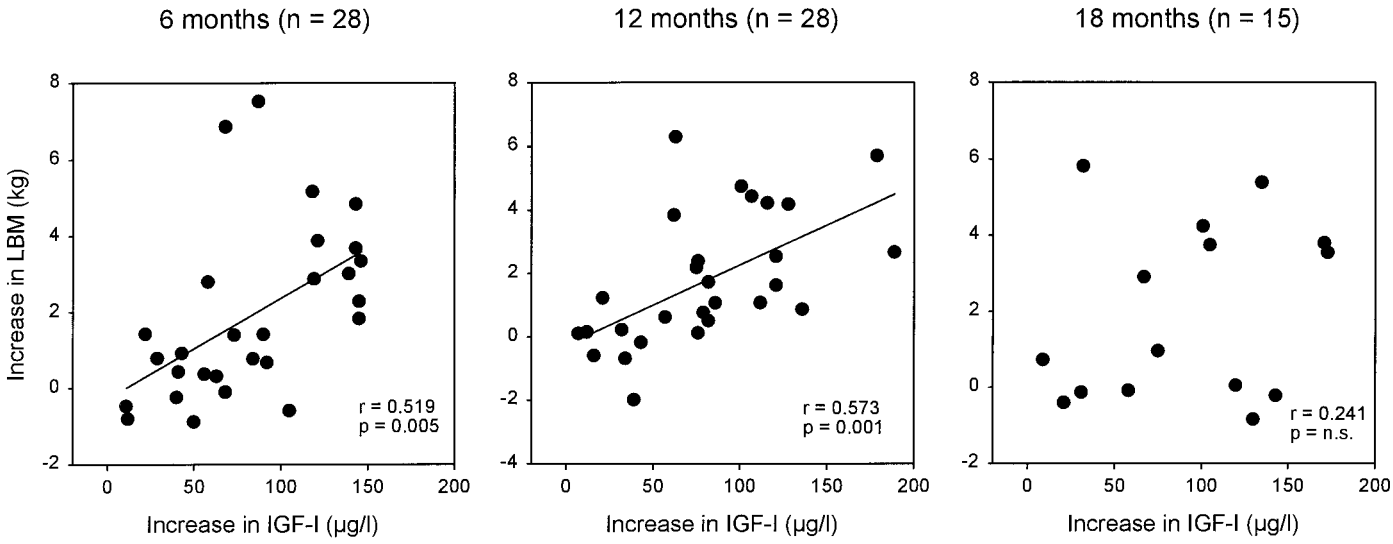

FIG. 4. Relationship between the increase in LBM and the increase in IGF-I during GH replacement therapy in patients with GHD, aged 60–79 yr.

subjects. Mean levels, expressed as the SD score of age-matched reference material, were −0.02 ± 0.27 in the lumbar spine, −0.17 ± 0.18 in the femoral neck, 0.16 ± 0.22 in the trochanter, and −0.33 ± 0.21 for the total body BMD. In the six female patients mean BMD values at the various sites were similar to those in the males. There was no significant relationship between BMD and the known duration of pituitary disease. When the BMD values in the patients were compared with those in young adults, 15 of 31 patients (48%) fulfilled the WHO definition for osteoporosis, *i.e.* a BMD below −2.5 SD score from the mean of young adults at any measured site. The range was −4.3–1.79 SD score for total body measurements, −4.4–0.43 at the femoral neck, −3.47–1.27 SD score at the trochanter, and −3.75–1.92 SD score at L2–L4. BMD did not change at any site measured during treatment. At baseline, trochanter BMD showed a correlation to IGF-I ($r = 0.37$; $P < 0.05$) and IGFBP-5 ($r = 0.37$; $P < 0.05$), whereas femoral neck BMD correlated to IGF-I only ($r = 0.38$; $P < 0.05$).

In the heel ultrasound measurements at baseline, the mean speed of sound was 1511 ± 6 m/s, and the broad band ultrasound attenuation was 111 ± 2 decibels/MHz. The stiffness index was −0.73 ± 0.23 SD score compared with age-matched controls, with a range between −3.14 and 1.26. Twelve of 25 patients (48%) had a stiffness index below −2.5 SD score from the mean of young adults. Individual stiffness indexes correlated to total BMD ($r = 0.64$; $P < 0.001$), lumbar spine BMD ($r = 0.39$; $P < 0.05$), femoral neck BMD ($r = 0.58$; $P < 0.01$), and trochanter BMD ($r = 0.72$; $P < 0.001$).

None of the ultrasound measurements changed significantly during treatment (not shown). The SOS correlated to IGF-I in serum ($r = 0.39$; $P < 0.05$) at baseline.

Normal values for all of the markers of bone metabolism were found at baseline, and no patient reached supranormal values during treatment. None of the markers changed in the placebo group during the 6-month placebo period, whereas a significant increase was found in the GH-treated patients (Table 2).

In the combined group (n = 28), mean values of the formation markers osteocalcin, PICP, and bone-specific ALP were significantly increased at 6, 12, and 18 months compared with baseline values (Fig. 4). The resorption marker urinary pyridinoline was significantly increased from baseline at 6 and 12 months, but not at 18 months (Fig. 5). The changes were similar in males and females.

Safety aspects and adverse events

One patient had impaired glucose tolerance at the start of the study and at 12 months, but not at 6 and 18 months. In another patient glucose tolerance was impaired at study start and at 6 months, and the subject was diabetogenic at 12 months (2 h blood glucose, 11.2 mmol/L), which was this patient's last visit. Of the patients with normal OGTT at the start of the study, one patient showed impaired glucose tolerance at 18 month only, and one patient had pathological blood glucose at 12 months (2 h blood glucose, 11.5 mmol/L).

Mild to moderate side-effects, probably due to fluid retention, were observed in eight patients during GH treatment and in one patient during placebo therapy, including peripheral edema, joint stiffness, and muscle pain, which all subsided spontaneously or after a minor dose reduction.

Discussion

In the present study the effects of GH replacement therapy on body composition and bone metabolism in elderly GHD patients are evaluated for the first time in a placebo-controlled manner. We found beneficial effects on body composition, with an increase in LBM and a decrease in TBF using GH doses leading to IGF-I levels within the normal range in the majority of the patients. The mean changes in LBM (1.9 kg) and TBF (−1.7 kg) at 6 months found in the present study are similar to those reported in a dose-finding study by Toogood and Shalet (33) in 12 GHD patients, aged 62–86 yr, treated with increasing GH doses; the highest dose was 0.5 mg/day, corresponding to 1.5 IU daily.

The mean changes in body composition seen in our patients, in particular concerning fat mass with unchanged

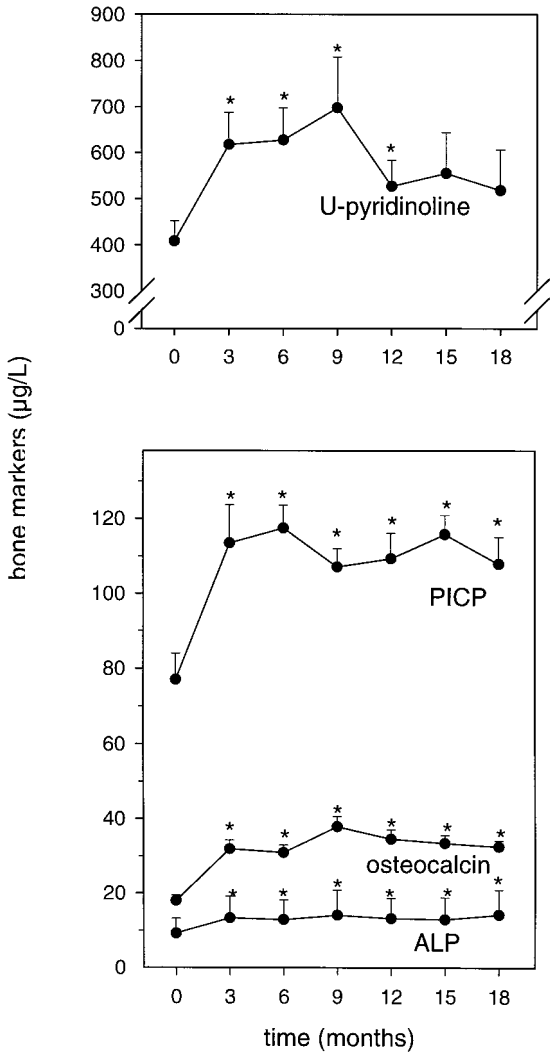

FIG. 5. Markers for bone metabolism (mean ± SEM) in serum (ALP, osteocalcin, PICP, and urinary pyridinoline) before and during GH replacement therapy (n = 28 for 0–12 months; n = 15 for 15 and 18 months) in patients with GHD, aged 60–79 yr. *, $P < 0.05$.

waist/hip ratio, are smaller than those found in previous studies in younger subjects. In general, a mean increase in LBM of 2–5.5 kg and a mean decrease in fat mass of approximately 4–6 kg were seen (1). However, the doses employed were often more than twice as high per kg BW as those used in our study, leading to unphysiologically high IGF-I serum concentrations (7, 8). In one study using dose titration to keep IGF-I concentrations within the normal range, the effect on body cell mass was similar to that found in the present study (34). Furthermore, it has been reported that LBM is not reduced in elderly GHD patients compared with healthy age-matched controls (35). Possibly factors other than GH may be relatively more important for the maintenance of LBM in the elderly GHD patients. They may also be more resistant to the lipolytic effect of GH, in view of the modest effect of GH therapy on adipose tissue in the present study.

When women and men in the present study were analyzed separately, it was found that only 2 of the 5 females had increased LBM at 12 months compared with 18 of the 23 males. The same dose per kg BW was given to men and women. It has previously been shown that females are less sensitive to GH replacement therapy than males, and the time until the effect occurs is longer (36–38). Regrettably, the number of females in the present study was too small to assess differences in response between gender statistically.

The limitations in measuring LBM by DXA should also be recognized. It is built on the assumption of a constant hydration of lean tissue, and fluid retention may lead to overestimation of LBM (39, 40). However, in the present study using low GH doses, the problems with fluid retention were mild or absent, and body weight did not change.

BMD values varied widely, but the mean levels were not different from those found in healthy age- and sex-matched individuals. This is in accordance with the findings of Toogood *et al.* (41). Previous studies have shown that BMD is preserved to a greater extent in adult-onset GHD compared with childhood-onset GHD (1).

BMD did not change during 12–18 months of treatment in our elderly GHD patients. In younger patients, a number of investigations found reduced BMD after 6–12 months, but an increase of 4–10% after more than 12 months has been demonstrated (1). Thus, a longer observation time is needed for a definite assessment of the effect on BMD. In addition, it has been shown that the gain in BMD is higher when the initial BMD is low (11). In our patients with normal BMD from the start of the study, only a modest increase in BMD can be anticipated even in the long-term perspective.

In the ultrasound measurements of calcaneus, the stiffness index was reduced below −2.5 SD from the mean of young adults in the same percentage of patients as with the DXA measurements, and there were significant positive correlations between stiffness index and BMD at all sites. Stiffness index was positively related to IGF-I levels. Ultrasound measures clinically relevant properties of bone strength distinct from bone density. *In vitro* studies have shown that broad band ultrasound attenuation values correlate with histomorphometric parameters of trabecular structure. In contrast, speed of sound measurements reflect elastic properties of bone (42). These variables were not altered by GH treatment in our patients. In one study phalangeal ultrasound transmission velocity showed an initial decrease followed by a return to baseline (43).

In contrast to the study by Toogood *et al.* (41), who found decreased levels of osteocalcin and deoxypyridinoline, indicating reduced bone turnover in elderly GHD patients, baseline values of markers for bone metabolism were normal in our patients. We do not know the reason for this. It may be due to differences in methodology and reference material.

We found that the markers for bone metabolism were significantly increased within 3 months of therapy, indicating activation of bone remodeling. The increase in bone formation markers was sustained throughout 18 months, whereas the bone resorption marker urinary pyridinoline was significantly elevated until 12 months. Increase in markers of bone metabolism has been consistently reported from trials in younger patients (1). We found in younger patients that the levels peaked at 6–12 months and had returned to baseline at 36 months (44). The magnitude of the increase in

our elderly patients appears similar to that in younger subjects.

In our patients, in addition to normalized IGF-I, there was a normalization of IGF-II and the GH-dependent IGFBPs, IGFBP-3 and IGFBP-5, during GH replacement therapy.

An age-related decline in circulating IGFBP-5 concentrations has been described in healthy subjects, possibly attributed to the declining GH secretion (13, 45). In contrast, IGFBP-4 concentrations tend to increase with age (27). In our GHD patients, IGFBP-4 levels also appeared higher in our older than in our previously studied younger subjects, with a mean of 418 ± 19 *vs.* 223 ± 23 μg/L (20). This indicates that an age-related rise in IGFBP-4 levels may also be present in GHD patients. Thus, a negative relationship between the two binding proteins is expected. We found, however, a positive correlation between IGFBP-4 and IGFBP-5 levels in our GHD patients. Possibly, the age-related pattern of IGFBP-5 is different when GH secretion is deficient. In addition, IGFBP-4 increased during GH therapy and that is in accordance with our findings in younger GHD patients. The physiological significance of the elevation of this binding protein, which is known to inhibit IGF actions, is unclear. It is established that IGFBP proteases play an important role in modulating IGFBP action (16). The antibodies used in the present study to measure IGFBP-4 detect both intact and fragment forms, and it is not known which forms are increased by GH treatment. Thus, the rise in IGFBP-4 can represent both synthesis and degradation. However, it has recently been found that IGFBP-4 given systemically in mice stimulates bone formation markers (46). A possible mechanism may be that more IGF-I can bind in a binary complex with IGFBP-4 and constitute a more readily available pool of IGF-I than the ternary complex with IGFBP-3 and the acid-labile subunit, which is confined to the intravascular space (16).

A decrease in IGFBP-1 was seen during the first 6 months. This probably reflected the increase in insulin, as IGFBP-1 is known to be negatively regulated by insulin (18, 19, 47). Over the whole treatment period, no significant alterations in IGFBP-1 were found. A similar pattern was seen with IGFBP-2. Although within the normal range in the present study, IGFBP-2 has been shown to be increased in GHD and decreased with GH replacement therapy (19).

As IGF-I mediates many of the anabolic effects of GH, and IGFBPs can modulate these effects, we investigated the relationships between LBM and serum IGFs and binding proteins. A positive correlation between LBM and IGF-I was found at baseline, 6 months, and 12 months. The increase in LBM was also correlated with the increase in IGF-I. IGF-I could thus be considered a marker for LBM changes during GH replacement. However, as some patients failed to respond with increase in LBM despite showing an increase in IGF-I, IGF-I does not seem to be an appropriate marker in the clinical setting. Relationships between LBM and serum IGFBP-1, -2, -3, -4, and -5 were not found. Thus, other markers for the changes in body composition should be sought.

IGFBP-5 is abundantly found in human bone and has been shown to stimulate IGF actions (18, 19). We have previously found positive correlations between BMD in femur (shaft and trochanter) and serum IGFBP-5 in younger GHD subjects (20). After long-term GH therapy when BMD had normalized, these associations were no longer found. In our older GHD patients with normal mean BMD, an association between trochanter BMD and IGFBP-5 was only found at baseline.

With the GH doses used (0.75–1.25 IU daily), serum IGF-I concentrations obtained were largely within the age-related normal range. It seems appropriate to administer a GH dose leading to IGF-I levels that do not exceed the upper physiological age-related range. It is possible that an IGF-I elevation above the normal range may have a greater impact in older than in younger patients due to their higher background risk of malignancy. In addition, population studies have indicated an association between serum IGF-I levels and prostate cancer risk, which has recently been reviewed (48). However, a causal relationship is not proven. IGFBP-3 is a strong inhibitor of IGF action in the prostate and even induces apoptosis (48, 49). With GH therapy, both IGF-I and IGFBP-3 increase, although the ratio of IGF-I/IGFBP-3 also increased in our patients. Patients with acromegaly have no increase in prostate cancer (48). As GHD patients often have low IGF-I, which normalizes during treatment, it does not seem likely at present that the cancer risk will increase above that in the normal population. Side-effects were few, mainly attributed to fluid retention, and subsided spontaneously or with minor dose reduction. Two patients who were normal at the start of the study had a deterioration of glucose tolerance.

In conclusion, elderly GHD patients respond to GH replacement therapy in a similar manner as younger patients with beneficial effects on body composition and increased bone metabolism. Although the increase in LBM correlated with the increase in IGF-I, the wide variation precluded IGF-I as a marker for change in LBM. There was no association between IGFBPs and LBM. With GH doses leading to IGF-I levels mostly within the age-related physiological range, side-effects were few, and elderly GHD patients can be considered for replacement therapy.

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