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The Effects of Growth Hormone Replacement Therapy on Bone Metabolism in Adult-Onset Growth Hormone Deficiency: A 2-Year Open Randomized Controlled Multicenter Trial*

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ABSTRACT

Adult hypopituitary patients with growth hormone deficiency (GHD) show a significant decrease in bone mass and an increased fracture rate. Replacement therapy with GH increases bone turnover. Most of the long-term data on bone mineral content (BMC) and bone mineral density (BMD) have been acquired in open, noncontrolled trials involving limited numbers of patients. To determine whether long-term GH therapy is beneficial for bone despite the increased bone turnover, 100 patients (59 men and 41 women), aged 25–65 years (mean, 49.7 years) with adult-onset GHD were randomized to treatment with GH (40 men and 28 women; mean dose, 0.18 IU/kg per week) or to a nontreated control group (19 men and 13 women) for 24 months. Despite a similar increase in parameters of bone turnover (osteocalcin [OC], procollagen type I carboxy-terminal propeptide [PICP], and pyridinolines ([PYD]) in male and female GH-treated patients compared with controls, the effects on BMC and BMD as evaluated by dual-energy X-ray absorptiometry were gender specific. A significant increase in spine BMC and BMD and total hip BMD and a decrease in BMD at the ultradistal radius over time was observed in male GH-treated patients compared with the evolution in controls (mean ± SEM change at 24 months: +6.8 ± 1.1% and $p = 0.009$, +5.1 ± 0.8% and $p = 0.005$, +3.5 ± 0.7% and $p = 0.02$, and −2.6 ± 0.8% and $p = 0.008$, respectively). No significant treatment effects were observed in female patients. Despite the increase in the total remodeling space induced by GH treatment, prolonged GH therapy in adult-onset GHD has a positive effect on bone balance, maintaining bone mass in women, and even increasing it in men over a 2 year-period. (J Bone Miner Res 2002;17:1081–1094)

Key words: adult, growth hormone deficiency, bone density, growth hormone therapy, controlled study

INTRODUCTION

GROWTH HORMONE (GH) has well-known effects on growth and development of the skeleton in children but also influences bone homeostasis in adults. GH-deficient (GHD) adults have a decreased bone turnover,[1,2] and GH replacement therapy in these patients clearly enhances both bone formation and resorption. GH excess in adults (acromegaly) increases bone size especially through periosteal bone growth. Acquired GHD in adults, either isolated or as part of panhypopituitarism, also has major adverse effects on body composition with increase in total fat mass and decrease in muscle and bone mass.[3] Based on cross-sectional and retrospective studies, such patients have a decreased quality of life (QoL), increased morbidity including an increased incidence of fractures, and higher mortality

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Table 1. Characteristics of Pituitary Disease

	Total	*GH group*	*Control group*
No. of patients	100	68	32
Men	59	40 (59%)	19 (59%)
Women	41	28 (41%)	13 (41%)
Mean age (years; range)	50	49 (25–65)	51 (28–65)
Etiology			
Pituitary adenoma	73	47 (65%)	26 (82%)
Nonfunctioning		27	20
Prolactinoma		11	4
Cushing's disease[a]		9	2
Craniopharyngioma	12	9 (13%)	3 (9%)
Other	15	12 (20%)	3 (9%)
IGHD	16	10 (15%)	6 (19%)
MPHD, treated for deficiency of	84	58 (85%)	26 (81%)
TSH	56	37 (54%)	19 (59%)
ACTH	53	34 (50%)	19 (59%)
FSH/LH	57	37 (54%)	20 (62%)
ADH	17	13 (19%)	4 (13%)
Mean peak GH (μg/liter; range)	1.3	1.4 (0.1–4.7)	1.2 (0.1–3.7)
0–1.99	81	56	25
2–2.99	13	7	6
3–4.99	6	5	1
Presumed duration of GHD (years; mean ± SD; range)	7.5 ± 6.5	7.0 ± 7 (1–36)	8.5 ± 6 (1–22)

Percentages (in parentheses) are in reference to each treatment group.
[a] Median duration postcure, 6.5 years (range, 1–22 years).

from cardiovascular disease.[3] GH replacement in GHD adults now has been accepted as a valuable addition to standard pituitary hormone replacement because of its favorable effects on body composition, improved well being, and reduction of several risk factors for diseases associated with GHD.[3]

A large number of short-term (6–12 months) controlled studies of GH replacement therapy have convincingly determined its capability to increase bone turnover but showed inconsistent results on bone mass.[1,2] Small-scale uncontrolled studies of prolonged GH replacement with follow-up up to 6 years suggested a progressive increase in bone mineral density (BMD).[4–12] However, up to now, only one controlled study of 18 months duration in adult GHD men has been published.[13] To evaluate the safety and long-term (i.e., spanning the normal remodeling cycle of bone by several times) effect of GH replacement therapy on bone mineral mass and BMD, we performed a prospective controlled 2-year study of 100 adult-onset GHD male and female patients of which ⅔ were treated randomly with GH and the remaining ⅓ was used as a control group. Thereafter, all patients were offered GH replacement therapy.

MATERIALS AND METHODS

Patients

One hundred hypopituitary patients (59 men and 41 women) with GHD acquired as adults and of at least 1 year duration were recruited in five Belgian centers (Table 1). None of them had been treated with GH before. Mean age was 50 years (range, 25–65 years) and suspected mean duration of GHD was 7.5 years (range, 1–36 years). Diagnosis of GHD was based on a peak serum GH response (assayed by polyclonal competitive radioimmunoassays (RIAs) of <5 μg/liter (at that time equivalent to10 mU/liter) after stimulation by either insulin-induced hypoglycemia ($n = 48$), GH releasing hormone (RH) ($n = 33$), or glucagon ($n = 19$) performed within 5 years before inclusion. All patients were white except for one Asian man. All patients had a history of adult-onset hypothalamic pituitary disease. In the majority of patients, pituitary deficiency was caused by a pituitary tumor: adenoma ($n = 73$) or craniopharyngioma ($n = 12$) and/or its treatment (surgery in 75 and radiotherapy in 33 patients). Pituitary tumor patients included 11 patients with inactive Cushing's disease and 15 patients with treated prolactinomas. Other causes of pituitary deficiency were Sheehan's syndrome (3), autoimmune hypophysitis (3), empty sella (3), posttraumatic (2), apoplexy (1), basal meningoencephalocele (1), idiopathic (1), and radiotherapy for cranial neurinoma (1). Eighty-four patients suffered from at least one additional pituitary HD (and 60 patients from two or more), and 16 patients had isolated GHD (IGHD) at inclusion. In the latter, diagnosis of IGHD had been confirmed by repeated provocative testing. Patients with multiple pituitary HD (MPHD) were on stable hormone-replacement therapy for at least 6 months. Deficiency of follicle-stimulating hormone (FSH)/

luteinizing hormone (LH), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), and antidiuretic hormone (ADH) was present in 74, 58, 55, and 17 patients, respectively. All 16 hypogonadal women under the age of 50 years were on estrogen-replacement therapy but only 5 of 18 gonadotrophin-deficient women aged ≥50 years were taking estrogens, as were the two postmenopausal women. Because of personal dislike, 4 of 40 hypogonadal men did not receive testosterone-replacement therapy before or during the study.

Patients with active metabolic disease capable of influencing bone metabolism, previous acromegaly, acute severe illness during the 6 months before inclusion, pregnancy, hepatic or renal impairment, diabetes mellitus, uncontrolled hypertension, malignancy, clinically significant cardiopulmonary disease, or chronic medication (except for hormone-replacement therapy, oral contraceptives, bromocriptine, cabergoline, antacids, H2-receptor-antagonists, proton-pump inhibitors, mild sedatives, low-dose aspirin, treatment for mild hypertension, or mild asthma) were excluded from the study. Pharmacologic doses of glucocorticoids were not allowed, but lipid lowering drugs and antiepileptic treatment were permitted, if started before and continued for the duration of the study.

Study design

Patients were enrolled into a 24-month prospective open randomized multicenter trial of either GH-replacement therapy or no treatment. Using a computerized procedure with seed number, a randomization list and sealed envelopes were produced by Pharmacia Corp. (Stockholm, Sweden), providing an uneven randomization with ⅓ of the patients into the nontreatment control group and ⅔ into the GH group, with gender stratification. Taking into account the clear effects of GH on bone in short-term double-blind studies, a placebo-controlled design implying 2 years of placebo injections was considered unethical. At the time of the study, reimbursement of GH-replacement therapy had not been obtained in Belgium.

During the first 4 weeks of treatment, the intended daily recombinant human GH (Genotropin; Pharmacia Corp.) dose was 0.02 IU/kg body weight (BW) per day (≈6.7 μg/kg) administered subcutaneously at bedtime. Thereafter, the intended dose was 0.03 IU/kg BW per day (≈10 μg/kg) with a maximum dose of 4 IU/day (≈1.33 mg), irrespective of BW. In case of adverse events, the dose could be reduced by stepwise dose reductions of 0.5 IU/day (≈0.17 mg) until an individualized dose was established. Dose titration according to insulin-like growth factor (IGF) I level was not part of the protocol. Drug compliance was assessed by vial count and monitoring of medication diaries and retrospectively by a rise in IGF-I during GH therapy. Intake of minerals, vitamins, or other supplements by either treatment group was not specifically recommended and remained unchanged for the duration of the study.

Bone mass measurements of the spine, the nondominant forearm, and the hip and total body composition were determined at baseline and every 6 months during the study period. A radiograph of the dominant hand was taken yearly. Laboratory examinations were performed at baseline; at 1-, 3-, and 6-month visits; and every 6 months thereafter. Blood samples were collected in the morning after an overnight fast. Physical examination and drug compliance were checked at 3-month intervals. Patients randomized to the control group had an identical follow-up.

All participants gave their written informed consent and the study was approved by the ethical committees of every participating hospital and was conducted in accordance with the guidelines proposed in The Declaration of Helsinki and with good clinical practice.

BMD

BMD of the lumbar spine (L2–L4), the left hip, the nondominant forearm, and the total body was measured by dual-energy X-ray absorptiometry. A GE Lunar DPX-L (GE Lunar Radiation, Inc., Madison, WI, USA) densitometer was used in one center and different types of the Hologic, Inc. QDR densitometer (Hologic, Inc. Waltham, MA, USA) were used in the other centers (a QDR-2000 in two centers and a QDR-4500 and a QDR-1000/W in one center each). The longitudinal follow-up of each patient was performed on the same equipment. In addition to a daily quality control of the equipment by spine phantom measurements as instructed by the manufacturer, the stability of the different densitometers was verified by yearly measurement of the ESP phantom 026. The CVs for precision of bone mineral content (BMC) and BMD measurements of the phantom for the densitometers used were ≤1.6% and ≤0.6%, respectively. Finally, before statistical analysis, an independent observer blinded to treatment allocation evaluated the quality of all scans, recalculating or omitting the data if the technical aspects of the longitudinal follow-up were not completely comparable.

BMC and BMD values, expressed in grams and grams per squared centimeter, respectively, are not directly comparable for Hologic, Inc. ($n = 73$) and GE Lunar Radiation, Inc. ($n = 26$) measurements because of different technical implementations.[14] Therefore, results of treatment are presented as percent changes from baseline.

The reference values provided by each manufacturer were used to calculate T scores, which express individual BMD values as SD scores in relation to the normal mean BMD values in a young normal population of the same gender. Patients were considered to be osteopenic when the T score was between -1 and -2.5 and were considered osteoporotic when the T score was < -2.5, according to the criteria defined by the World Health Organization (WHO).[15] Age-, gender-, and (GE Lunar only) weight-adjusted Z scores were used for the evaluation of BMD loss attributable to the GHD state.

In addition, cortical bone thickness was assessed yearly by radiogrammetry.[16] All calculations were performed by a single observer. The metacarpal index is the percent ratio of cortical area of the metacarpal to the square of periosteal diameter $(D^2 - d^2)/D^2$ and corrects for differences in skeletal size. The reproducibility of the method is 3.1%. Normal values are age-, gender-, and menopause-related.[17]

Body composition, muscle strength, and QoL

Dual-energy X-ray absorptiometry, using the equipment described previously, was used to measure lean body mass (LBM) and percentage of body fat. Muscle strength was evaluated by measuring the grip strength of the dominant hand with the Jamar hand dynamometer as the average of three attempts following the instructions of the manufacturer. QoL was evaluated using an adult GHD disease–specific questionnaire Quality of Life Assessment of GHD in Adults (QoL-AGHDA).[18] This questionnaire provides information on a range of physical, emotional, and social issues, relevant to patients with GHD. This QoL-AGHDA consists of 25 yes/no items. The total score is a simple summation of the number of "yes" responses, with a high score indicating greater distress (poor QoL). Baseline values (mean score of two questionnaires, taken at a 4-week interval) were obtained before patients were informed of their treatment assignment.

Markers of bone turnover

Biochemical markers of bone turnover were analyzed in a subgroup of 41 patients (28 treated patients and 13 control patients) from two centers. Fasting serum specimens and urine samples from an acidified 24-h urine collection were stored at −20°C immediately after sampling and all measurements of each individual were performed using the same batch of assay reagent. Urinary pyridinolines (PYDs) and deoxypyridinolines (DPDs) normalized for urinary creatinine, markers of bone resorption, were measured in 24-h urine collections acidified with hydrochloric acid, by high-performance liquid chromatography (HPLC) as previously described.[19] Values are expressed as micromoles per mole of creatinine, and urinary creatinine was measured colorimetrically.[20] Interassay CVs were 11.5% and 13.3% for PYD and DPD, respectively, and the intra-assay CVs were 10.2% and 12.5%. Normal values were 22–89 μmol/mol and 4–21 μmol/mol of creatinine for PYD and DPD, respectively. Serum osteocalcin (OC) levels were measured by a homologous human OC RIA. The within- and between-assay CVs were 4.5% and 8.6% for a low value (7.6 μg/liter) and 6.4% and 7.1% for a high value (36 μg/liter), respectively; the mean (±SD) values in adults were 25 ± 5 μg/liter in men, 20 ± 6 μg/liter in premenopausal women, and 29 ± 2 μg/liter in postmenopausal women.[21] Serum procollagen type I carboxy-terminal propeptide (S-PICP) levels were measured by RIA (Orion Diagnostica, Espoo, Finland). The between-assay variation coefficient was 4.1% at 105 μg/liter and 6.6% at 216 μg/liter; the within-assay variation was 2.1% at 103 μg/liter and 3.2% at 451 μg/liter. The normal ranges were 38–202 μg/liter and 50–170 μg/liter in men and women, respectively. Serum intact parathyroid hormone (PTH) levels were measured by a human PTH [hPTH(1-84)] immunoradiometric assay (IRMA) with polyclonal and monoclonal region–specific antibodies.[22] The within-assay CVs were 2.4%, 3.7%, and 8.1% for high (108 ng/liter), medium (20 ng/liter), and low (10 ng/liter) PTH(1-84) concentrations, respectively. The between-assay CVs were 6.7% and 6.8% for mean PTH(1-84) concentrations of 21 ng/liter and 53 ng/liter, respectively; the normal range was 3–30 ng/liter. Serum 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] levels were measured by RIA after extraction, as previously described.[23] The between- and within-assay CVs at a concentration at 42 ng/liter were 14% and 11%, respectively. The mean (±SD) value in healthy adults is 38 ± 12 ng/liter. Serum calcitonin (CT) levels were measured by IRMA (Medgenix Diagnostics, Fleurus, Belgium). The intra-assay and interassay CVs were 3.1% and 5.3% at 38.3 ng/liter. The normal range in an adult population was 0–15 ng/liter. Finally, urinary calcium and phosphorus were assayed centrally in this subgroup of patients, and serum calcium, phosphorus, creatinine, and alkaline phosphatase (sALP) were assayed in all patients using standard methods in the five local laboratories.

Other biochemical assays

The serum concentrations of IGF-I and IGF binding protein (BP) 3 (IGFBP-3) in all patients were measured by Pharmacia Corp. using RIA after acid-alcohol extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) and ELISA (Diagnostic System Laboratories, Webster, TX, USA), respectively. Analysis, using frozen serum samples, was performed after completion of the study. IGFBP-3 results were expressed in milligrams per liter and those of IGF-I were expressed in micrograms per liter and as a SD score comparing the individual result with those of an age-matched reference population [395 randomly selected healthy Swedes from the Monica study[24]: algorithm, SDs = (ln(IGF-I) − (5.92 − 0.0146 × age))/0.272].

Statistical analysis

The primary efficacy variable was the mean relative change of BMC at the lumbar spine (L2–L4) after 24 months of treatment. Based on a relevant improvement of 2% per year of the L2–L4 BMC in the GH-treated group with 90 evaluable patients randomly assigned, 30 to the control group and 60 to the treatment group, at the end of the 2-year period, the power of the study was 80% at a 5% significance level. Secondary objectives were the evaluation of the mean relative change of BMD at the lumbar spine, the hip, and the forearm of total body BMC and body composition.

The effect of GH over time (6, 12, 18, and 24 months) on BMC and BMD was analyzed using a linear mixed model.[25] Presence of a gender difference was evaluated. The p values were corrected for multiple testing using the step-down Bonferroni method of Holm.[26] Statistical significance was assumed for $p < 0.05$. Analyses were performed using the SAS version 6.12 TS 051. Data were analyzed on an intention-to-treat basis. Two patients in the treated group were excluded from the efficacy analysis because of protocol violations (a pharmacologic glucocorticoid replacement dose and start of oral methylprednisolone for emphysema, respectively).

TABLE 2. BASELINE CLINICAL CHARACTERISTICS IN GH-TREATED AND CONTROL PATIENTS

	Males		*Females*	
	GH	*Control*	*GH*	*Control*
No. of patients	40	19	28	13
Mean age (years)	49 ± 11	52 ± 11	50 ± 11	49 ± 13
Height (cm)**	174 ± 8	174 ± 7	162 ± 6	163 ± 5
Weight (kg)	84 ± 13	83 ± 9	81 ± 18	78 ± 11
BMI (kg/m^2)*	27.8 ± 3.6	27.4 ± 2.5	31.3 ± 7.4	29.2 ± 5.1
% BF (% of BW; DXA)**	29 ± 7	28 ± 5	45 ± 9	44 ± 7
LBM (kg; DXA)**	56 ± 9	56 ± 7	41 ± 7	40 ± 3
Waist (cm)	98 ± 10	97 ± 6	97 ± 16	94 ± 10
Waist/hip ratio**	0.96 ± 0.06	0.95 ± 0.04	0.86 ± 0.06	0.87 ± 0.06
Hand power (dynamometry)	46.7 ± 8.6	46.8 ± 7.4	27.1 ± 4.8	30.7 ± 5.6
Metacarpal index	80.6 ± 6.9	79.1 ± 8.5	80.7 ± 7.8	84.4 ± 10.3
QoL-AGHDA-score**	7.6 ± 5.2	8.2 ± 5.7	12.9 ± 6.5	12.0 ± 7.0
IGF-I (μg/liter)**	177.5 ± 76.2	137.7 ± 66.0	104.3 ± 60.4	92.3 ± 57.0
IGF-I SDs**	−0.4 ± 1.6	−1.4 ± 1.9	−2.8 ± 2.7	−3.2 ± 2.7
IGFBP-3 (ng/ml)	3.14 ± 0.74[a]	2.62 ± 0.74	2.89 ± 1.19	2.65 ± 0.72

Results are the mean ± SD.

BF, body fat; BMI, body mass index; LBM, lean body mass; DXA, dual-energy X-ray absorptiometry.

Asterisks indicate significant differences between genders: * $p = 0.005$; ** $p < 0.0001$.

[a] GH-treated males had higher baseline IGFBP-3 than nontreated males ($p = 0.014$), $p > 0.05$ for all other comparisons between GH and control groups of same gender.

RESULTS

Baseline characteristics, compliance, and tolerance

Clinical characteristics at baseline did not differ between patients in the GH and the control group, as shown in Table 2. Ninety-two patients completed the study, of whom 62 patients were in the treated group and 30 patients were in the control group. Three GH-treated patients withdrew within the first 4 months because of GH-related fluid retention effects (joint pain and edema with or without carpal tunnel syndrome, respectively). Reasons for study discontinuation during the second year of treatment were performance of gastric banding surgery, emphysema, and noncompliance. Reasons for discontinuation in the control group were pregnancy and a demand for GH-replacement therapy. Overall, compliance in the 62 patients completing the study was very good. Noncompliance, defined as missing 15% of injections or more (one injection per week) was present in 4 patients but was caused by treatment interruptions of 3–9 months duration in 3 of these patients. Reasons for interruption were arterial hypertension, a rise in serum creatinine (both noncorrecting after discontinuation), and treatment fatigue. Only three of the 58 compliant patients had missed >5% of the injections.

Adverse events possibly related to fluid retention were reported in 51 (75%) treated patients versus 5 (15%) control patients (χ test, $p < 0.0001$): one or more episodes of arthralgia (in 53% vs. 12.5% of controls; $p = 0.0001$), edema (41% vs. 3%; $p < 0.0001$), paresthesia including the carpal tunnel syndrome (31% vs. 3%; $p = 0.002$), and/or myalgia (21% vs. 3%; $p = 0.02$) occurred significantly more frequently in the GH-treated group. There was no difference in the reported frequency of other adverse events, respiratory disorders (in 47% vs. 50%, respectively) being the most common. The majority (>70%) of the fluid-related adverse events in the GH-treated group occurred within the first 6 months of GH treatment. There was a nonsignificant gender difference in the occurrence of fluid retention–related side effects (in 82% of women vs. in 70% of men) explaining that women needed transient or permanent reductions of their GH dose more frequently (64% of women vs. 42.5% of men; $p = 0.08$).

GH-dose and IGF-I, IGF-I sodium dodecyl sulfate, and IGFBP-3

Dose reductions from the intended 0.21 IU/kg per week (≈10 μg/kg per day) were required in over one-half of the patients. The maximum mean dose of GH was 0.19 ± 0.03 IU/kg per week (≈9 ± 1.4 μg/kg per day) at 3 months in women and 0.19 ± 0.04 IU/kg per week (≈9 ± 1.8 μg/kg per day) at 6 months in men. Thereafter and for the duration of the second treatment year, the mean dose of GH was 0.17 ± 0.05 IU/kg per week (≈8.1 ± 2.4 μg/kg per day) in women and 0.18 ± 0.05 IU/kg per week (≈8.5 ± 2.4 μg/kg per day) in men, equivalent to a daily injected dose of 2 ± 0.6 IU (≈0.67 ± 0.2 mg) in both genders.

Baseline IGF-I and IGF-I SDs did not differ between treatment groups (Table 2) but were significantly lower in female patients than in male patients (mean, IGF-I ± SD of 100 ± 59 μg/liter vs. 165 ± 75 μg/liter, respectively; $p < 0.0001$). The percentage of patients in the entire study group at baseline with a low IGF-I (SD < −2) was 61% in women and 24% in men. Four men (7% of men, all MPHD and randomized to the GH group) had high IGF-I (SD > +2) at baseline. There was a significant increase in serum IGF-I

with GH treatment for the duration of the study (Fig. 1). The percentage of patients with supraphysiological serum IGF-I concentrations (SD $>$ +2) at 24 months who had low (SD $<$ −2) or normal (SD ± 2) pretreatment levels was 21% and 50% in women and 40% and 83% in men, respectively.

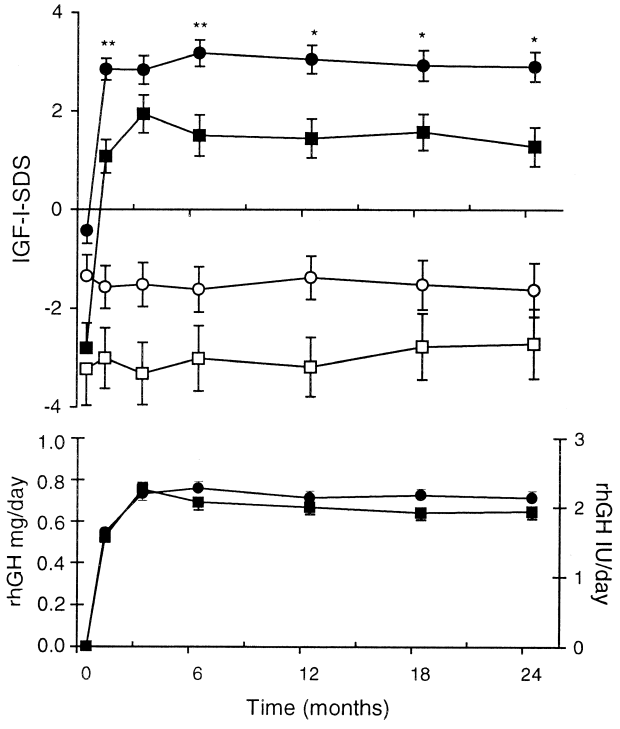

FIG. 1. Mean IGF-I SDs (absolute value) in male (●) and female (■) GH-treated patients and in male (○) and female (□) control patients in relation to the mean GH dose in the two treated groups. Error bars represent ± SEM. $p < 0.0001$ for all comparisons of IGF-I SDs during GH treatment between the GH-treated groups and the control groups. $*p < 0.01$ and $**p < 0.001$ for differences in IGF-I SDs with GH treatment between the male- and female-treated groups.

Serum IGFBP-3 concentrations sharply increased from a mean ± SEM of 3.04 ± 0.12 ng/ml at baseline to 4.08 ± 0.11 ng/ml at 1 month in the GH group and plateaued thereafter, whereas the control group showed no change ($p < 0.0001$). There was no gender difference for IGFBP-3.

Baseline BMD

The mean Z score was significantly different from zero, indicating a decrease in BMD in GHD patients compared with their age- and gender-matched controls, at all measured sites in men and at the lumbar spine and the hip in women aged <50 years. This reduction persisted after exclusion of the 4 men with untreated gonadal insufficiency (Fig. 2) and of the patients with a previous history of ACTH- or prolactin (PRL)-producing tumors (not shown). In contrast, BMD in all women was normal at the forearm. Women aged ≥50 years, despite a nonsubstituted hypogonadism in 13 out of 20, showed normal bone density for their age at the lumbar spine and at the hip as well.

At least osteopenia (T score < -1 SD) was present at the lumbar spine in 52% and 50%, at the femoral neck in 71% and 63%, at the total hip in 51% and 50%, at the ⅓ forearm in 47% and 33%, and at the ultradistal forearm in 53% and 23% of men and women, respectively.

The mean (±SEM) metacarpal index expressed as an SD score relative to a gender- and decade-specific mean was 0.09 ± 0.21 and 0.01 ± 0.22 in female ($n = 28$) GHD patients and male ($n = 43$) GHD patients, respectively (NS).

Effect of GH on bone metabolism

The effects of GH treatment on serum bone formation and urinary bone resorption markers are shown in Fig. 3. The serum OC concentration doubled during the first 6 months

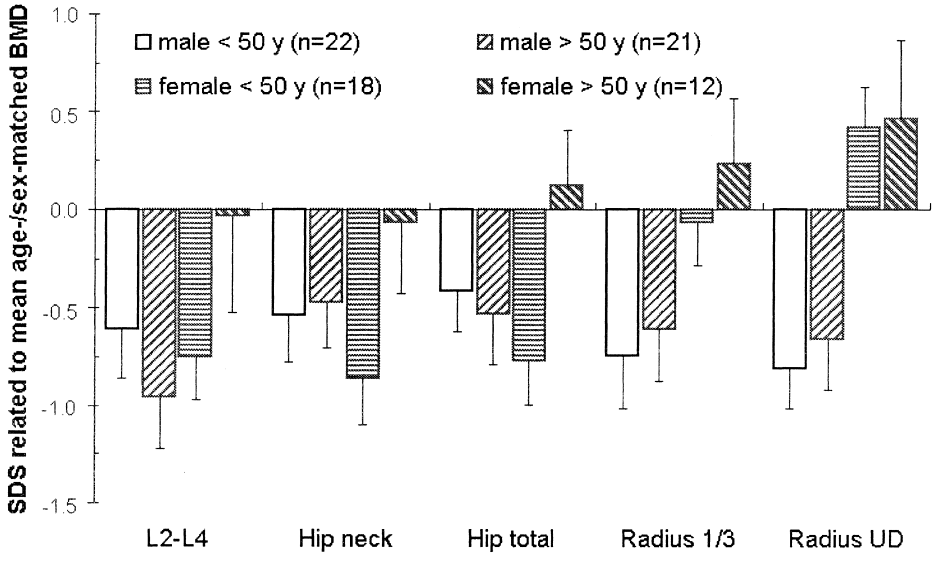

FIG. 2. Baseline Z score in GHD patients, stratified according to age and gender (Hologic, Inc.; measurements only; $n = 73$). Values are mean ± SEM. The value of $p < 0.05$ for all measured sites in male patients and for the lumbar spine, femoral neck, and total hip in female patients <50 years old.

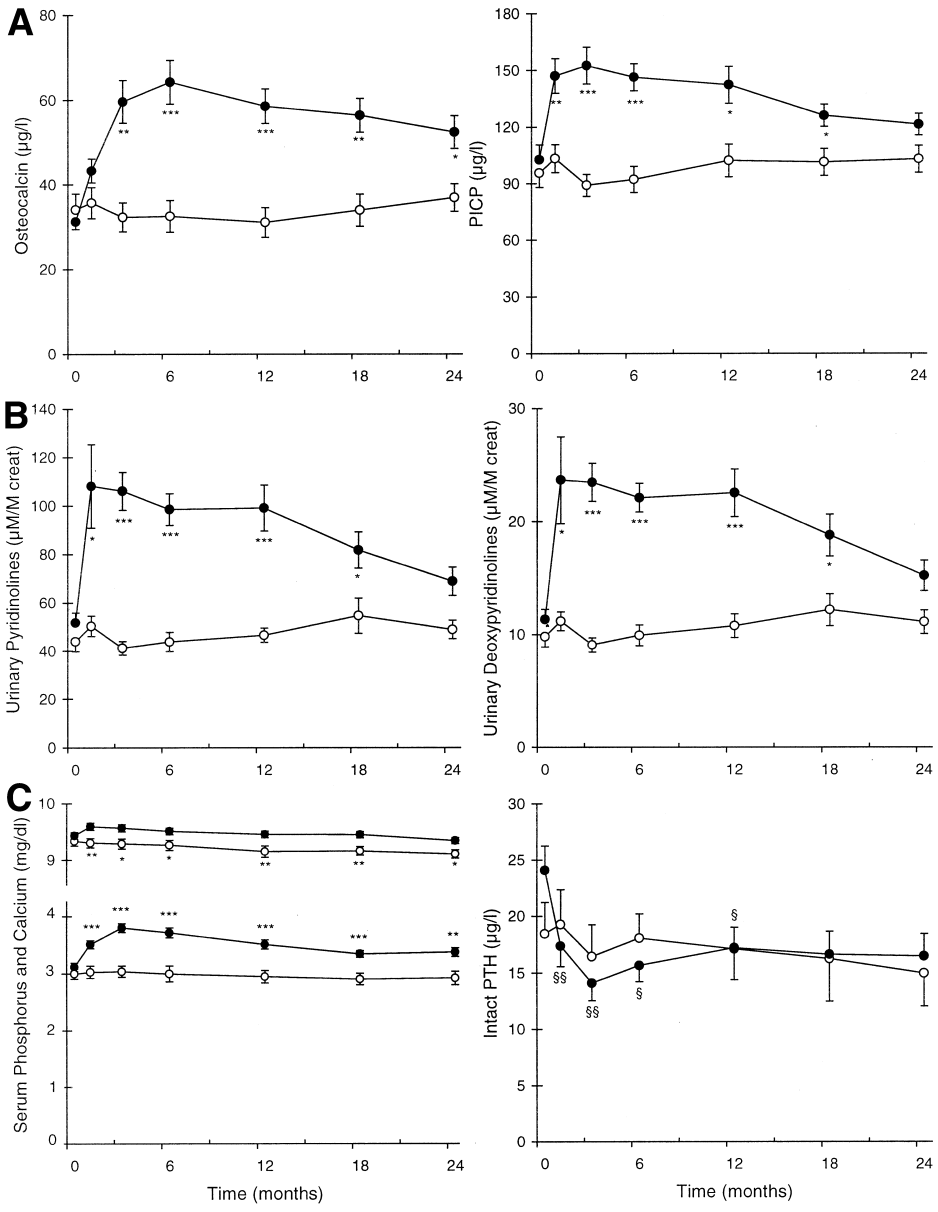

FIG. 3. Evolution of (A) serum bone formation markers ($n = 41$), (B) urinary bone resorption markers ($n = 38$), and (C) serum calcium, phosphorus ($n = 100$), and PTH ($n = 41$) in GH-treated (●) and control (○) patients over the 2-year period. Error bars represent ±SEM $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ for differences between the groups. §$p <$ 0.05 and §§$p < 0.01$ for differences in change from baseline between the groups.

of GH treatment from 31 ± 9 µg/liter to 64 ± 5 µg/liter ($p < 0.00001$ vs. controls) and gradually decreased thereafter but remained significantly elevated at 24 months at 52 ± 4 µg/liter ($p < 0.01$ vs. controls). Serum PICP values showed a similar evolution with a maximum at 3 months (from 103 ± 8 µg/liter to 153 ± 10 µg/; $p < 0.0001$ vs. controls) and a persistent elevation for the duration of the study, although at 24 months, the value (121 ± 6 µg/liter) was no longer significantly different ($p = 0.07$) from the control group. sALP concentration increased with GH treatment only in men (from 103 ± 8 IU/liter to 118 ± 10 IU/liter at 6 months; $p < 0.05$ vs. all other groups) and returned to baseline levels during the second treatment year whereas in both controls and GH-treated women, sALP significantly declined from 12 months onward (not shown). The increase of urinary PYD and DPD was maximal at 1 month of GH treatment (from 52 ± 4 µmol/mol to 108 ± 17 µmol/mol of creatinine and from 11 ± 1 µmol/mol to 24 ± 4 µmol/mol of creatinine, respectively) and persisted for 1 year ($p < 0.0001$ at 3, 6 and 12 months vs. controls) whereafter the change from baseline no longer significantly differed between the two treatment groups.

The serum calcium level increased by a mean of 0.18 ± 0.06 mg/dl at 1 month of GH treatment ($p < 0.01$ vs. controls) and remained higher than in the control group for the duration of the study. These effects were even more pronounced for the serum phosphate level that was significantly higher with GH treatment ($p \leq 0.001$ vs. controls) at all time points with a maximal increase of 0.69 ± 0.08 mg/dl at 3 months. There was a significant decrease in serum PTH concentration during GH treatment with a maximal decrease of −10.0 ± 1.6 pg/ml at 3 months ($p < 0.01$

vs. controls), persisting at 18 months and 24 months ($p < 0.001$ compared with baseline) although the ΔPTH no longer differed from the control group. Serum CT levels remained unchanged whereas the $1,25(OH)_2D$ concentration did not show consistent changes (not shown). Urinary calcium excretion in male patients increased only transiently in response to GH treatment (from 102 ± 19 mg/g to 151 ± 25 mg/g of creatinine at 1 month; $p < 0.01$ compared with controls), whereas in female patients the increase was more pronounced (from 124 ± 21 mg/g to 219 ± 39 mg/g creatinine at 3 months; $p < 0.001$ vs. controls) and persisted over the 2-year period ($p < 0.05$ vs. male treated patients). No gender differences in response to GH treatment were observed unless otherwise indicated.

Effect of GH on bone mass and density

The effects on BMC and BMD of GH administration for 2 years are shown in Table 3 and the significant effects are illustrated in Fig. 4. A significant difference in evolution between the GH-substituted and the control groups was observed at the lumbar spine (BMC, $p = 0.036$; BMD, $p = 0.006$) and at the ultradistal radius (radial BMD, $p = 0.004$). The difference in spine BMC and BMD over time was significant in men only, after 24 months of GH therapy (BMC, $p = 0.009$; BMD, $p = 0.005$, for the treatment effect in men). The mean ± SEM increase in spine BMC and BMD in GH-treated male patients at 2 years was 6.8 ± 1.1% and 5.1 ± 0.8%, respectively. In women, administration of GH for 2 years did not result in a significant treatment effect for the spine BMC ($p = 0.87$), whereas a significant treatment effect for spine BMD ($p = 0.046$) was caused by a transient decrease in BMD in the control group at 6 months. Although female and to a lesser degree male GH-treated patients showed a progressive decline in ultradistal (UD) radial BMD compared with baseline (−5 ± 1.6% and −2.6 ± 0. 8% at 24 months, respectively), the evolution in GH-treated women did not significantly differ from their controls ($p = 0.08$; $p = 0.008$ for treatment effect in women and men, respectively). Female GH-treated patients showed a less pronounced decline in BMD at the distal ⅓ radius as well, although once again, no difference with controls could be shown.

Total hip BMD, although not statistically significant for the total group (treatment effect, $p = 0.057$), showed a significant progressive increase in GH-treated males (+3.5 ± 0.7% at 24 months, $p = 0.019$ for treatment effect in men). The evolution of bone density measurements at the other sites (femoral neck BMD and total body BMC) did not show any treatment or gender effects. The control group did not show any significant changes throughout the 24 months of the study. Patients with lower initial bone mass (Z score < −1 SDs) did not show more marked increases in BMD.

Among the 33 male patients who completed the 24 months of GH treatment, the percentage with at least osteopenia decreased from 52 to 36% at the lumbar spine, from 79 to 70% at the hip, from 54 to 50% at the femoral neck, was unchanged (53%) at the ultradistal radius, and increased from 38 to 47% at the proximal radius (all NS). In the female GH-treated group, no significant changes in the number of patients with at least osteopenia were observed. The metacarpal index, evaluated in 59 patients, did not change significantly over time.

Effect on other measures: body composition, muscle strength, and QoL

At the end of the 2-year period, both GH-treated and control patients had increased their BW by a mean of 1.5 ± 0.7 kg. GH administration resulted in a significant decrease in body fat and an increase in lean mass. As shown in Fig. 5, these beneficial changes in body composition were initially greater in male GH patients compared with female GH patients, but the gender difference lessened over time. In contrast, only GH-treated women showed a significant increase in grip strength from 12 months onward (+8.3 ± 3.8% at 18 months; $p < 0.05$).

Female patients participating in the protocol experienced greater psychological distress as shown by a higher mean baseline QoL-AGHDA score (12.7 ± 1.0 vs. 7.8 ± 0.7 in men; $p < 0.001$). However, GH treatment was associated with a similar improvement of the QoL-AGHDA score in both genders, which was significantly different compared with nontreated controls from 18 months onward (mean decrease in score of 3.5 ± 0.8 and 4.5 ± 0.8 at 18 months and 24 months, respectively, vs. no change in controls; $p < 0.001$).

DISCUSSION

We examined the effects of GH-replacement therapy on bone in the largest population of adult-onset GHD patients studied so far. By including patients with adult-onset GHD only, a randomly selected GHD control group for the total duration of the study and a sufficient number of patients of both genders, we were able to deal with several of the limitations of previous studies.

Bone density at baseline was decreased in GHD patients at the lumbar spine (−0.4 Z score), femoral neck (−0.5 Z score), total hip (−0.5 Z score), and distal forearm (−0.4 Z score). These data are in line with previous data in similar[27,28] or smaller[29–31] groups of patients aged 55 years or less with adult- or mixed-onset GHD and indicate that bone remodeling, even at a lower rate than normal,[32] is sufficiently imbalanced to produce a small but significant loss of BMD. Whether this is caused by a direct effect of GH/IGF-I on bone cells or mediated by the GH/IGF-I effects on other body tissues is unclear. Usually, obesity is associated with an increased bone mass. Despite their absolute and relative increase in fat mass, GHD adults make an exception to this rule. The associated relative decrease in LBM or muscle mass could be an indirect mechanism contributing to their lower bone mass.

The decreased baseline BMD was observed in men as well as in premenopausal women at weight-bearing sites but not in postmenopausal women. Others[33,34] also have observed that elderly subjects with acquired GHD have no significant bone deficit at the time of diagnosis. The reasons

TABLE 3. EVOLUTION OF BMD (ESTIMATED MEAN PERCENT CHANGE ± SEM) AT EACH TIME POINT ACCORDING TO TREATMENT GROUP AND GENDER

	Male patients				*Female patients*			
Duration of treatment	*6 Months*	*12 Months*	*18 Months*	*24 Months*	*6 Months*	*12 Months*	*18 Months*	*24 Months*
BMC L2–L4								
GH group	0.81 ± 0.61	2.64 ± 0.82†	4.26 ± 0.84††	6.79 ± 1.05**	−0.50 ± 0.97	−0.97 ± 1.14	0.75 ± 1.10	1.06 ± 0.99
Control group	1.43 ± 0.97	2.90 ± 1.67	2.44 ± 1.09	1.72 ± 1.03	−1.65 ± 0.99	−1.18 ± 1.00	−0.41 ± 1.15	−0.03 ± 1.35
BMD L2–L4								
GH group	0.12 ± 0.48	2.05 ± 0.62†	3.43 ± 0.68††	5.09 ± 0.83**	0.30 ± 047*	−0.42 ± 0.59	0.84 ± 0.77	1.41 ± 0.78
Control group	−0.29 ± 0.94	1.28 ± 1.09	1.05 ± 0.63	0.81 ± 0.73	−2.36 ± 0.57	−1.60 ± 0.94	−0.97 ± 0.81	−0.23 ± 0.78
Femoral neck BMD								
GH group	−1.40 ± 0.74	−0.14 ± 0.87	−1.44 ± 0.83	2.25 ± 0.91	0.65 ± 0.57	1.60 ± 0.75	0.46 ± 0.89	2.14 ± 1.20
Control group	−3.09 ± 1.38	−1.97 ± 1.98	0.84 ± 0.84	0.29 ± 1.06	0.32 ± 1.24	1.26 ± 1.36	−0.34 ± 1.73	0.49 ± 1.63
Total hip BMD								
GH group	0.24 ± 0.73	1.17 ± 0.50	2.87 ± 0.64**	3.49 ± 0.69**	−0.39 ± 0.51	0.48 ± 0.63	−0.23 ± 0.72	1.54 ± 0.64
Control group	−0.43 ± 0.68	−0.75 ± 0.76	−0.82 ± 0.83	0.14 ± 0.90	0.50 ± 0.66	0.08 ± 0.81	−0.52 ± 1.07	0.79 ± 1.25
Radius 1/3 BMD								
GH group	−0.43 ± 0.28	−0.98 ± 0.38	−1.19 ± 0.40	−1.01 ± 0.43	−0.52 ± 0.46	−1.58 ± 0.47†	−2.25 ± 0.54‡	−1.76 ± 0.65
Control group	−0.19 ± 0.38	−0.47 ± 0.38	−0.66 ± 0.43	−0.71 ± 0.31	0.13 ± 0.66	0.25 ± 0.71	−0.08 ± 0.84.	0.11 ± 0.87
Radius UD BMD								
GH group	−2.61 ± 0.53***	−2.92 ± 0.77*	−3.24 ± 0.74**	−2.58 ± 0.78*	−1.22 ± 0.98	−3.24 ± 1.47	−4.97 ± 1.55†	−5.03 ± 1.58†
Control group	1.74 ± 0.88.	0.53 ± 0.71	0.58 ± 0.77	0.49 ± 0.54	−1.41 ± 0.59	−0.42 ± 1.32	−1.78 ± 0.83	−3.11 ± 1.27
BMC total body								
GH group	−0.53 ± 0.33	−0.61 ± 0.50	1.08 ± 0.74	1.09 ± 0.72	−1.47 ± 0.59	−1.13 ± 0.63	−0.55 ± 0.74	−1.49 ± 0.93
Control group	0.87 ± 0.54	0.68 ± 0.47	1.28 ± 0.43	1.01 ± 0.56	−0.03 ± 0.46	0.43 ± 0.60	0.58 ± 0.67	−0.04 ± 1.08

Significant differences in evolution between male and female GH-treated patients were observed for spinal BMC ($p = 0.003$), spinal BMD ($p = 0.003$), and radial UD BMD ($p = 0.012$).

Asterisks indicate statistical significant changes compared with controls of same gender: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Daggers indicate additional significant changes (with correction for multiple testing) versus baseline: † $p < 0.05$; ‡ $p < 0.01$; †† $p < 0.001$.

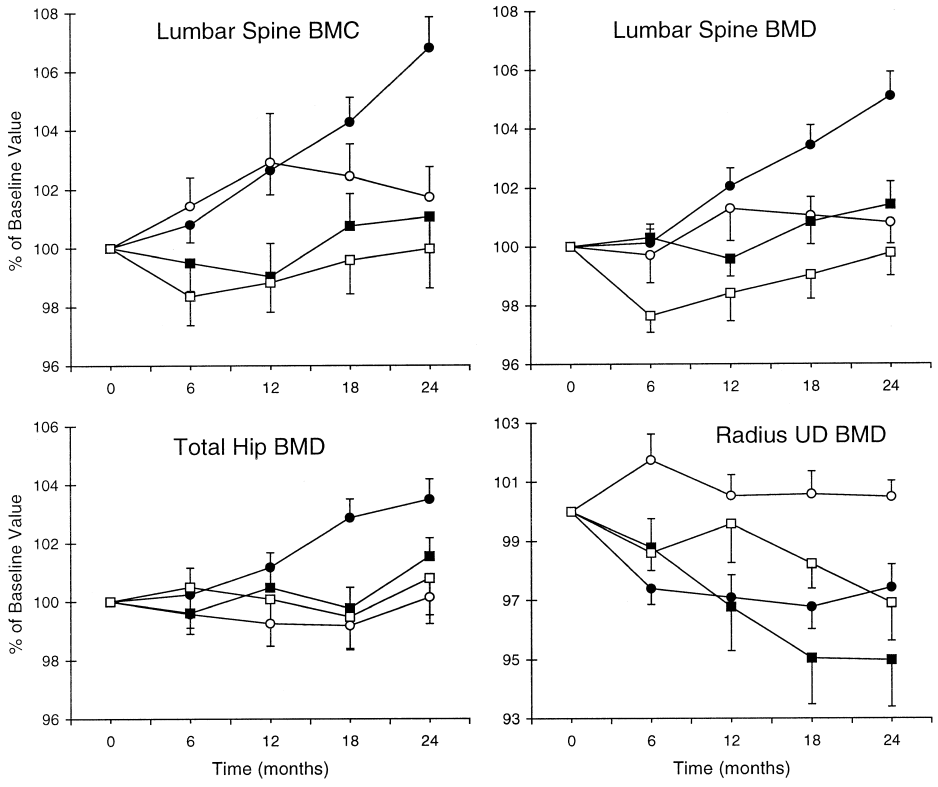

FIG. 4. Evolution of BMC/BMD at three sites according to treatment group and gender. ●, male GH-treated; ■, female GH-treated; ○, male controls; □, female controls. Values are the percentages of the baseline values; error bars represent ± SEM. The p values for the treatment effect in male patients were $p = 0.009$, $p = 0.005$, $p = 0.019$, and $p = 0.008$ for spine BMC, spine BMD, total hip BMD, and radial BMD, respectively.

are not well understood but the important bone loss generated by estrogen deficiency may mask the less important GH-mediated bone loss in postmenopausal women. The degree of osteopenia observed should predispose adult GHD patients to a 1.5- to 2-fold increased risk of fracture, if bone quality and other risk factors are the same as in prospective controlled studies.[35] Two small-scale cross-sectional studies of adult-onset GHD patients[36,37] have suggested such an increased prevalence of fractures. Moreover, the Pharmacia Corp. International Metabolic Database KIMS, a large-scale pharmacoepidemiological survey, also indicated an increased fracture risk in adult GHD patients.[38]

The "normal" baseline IGF-I values in 39% of women and 76% of men are not unexpected in this older group of patients with adult-onset GHD. In a similar population of adult-onset GHD patients (mean age of 45 years), 70% of IGF-I and 72% of IGFBP-3 estimations were within the range of normal subjects.[39] The time of onset of GHD[40,41] and the age of the patient[42,43] are two factors determining baseline IGF-I, with significantly lower values in childhood-onset GHD and at a lower age (<40 years), resulting in an increasing overlap between GHD patients and the normal range with age.

Both male and female patients responded to the GH treatment with the expected changes in body composition.[3] However, women showed a less pronounced decrease in body fat percentage during the period of 0–18 months, in keeping with previous observations in a 9-month crossover study,[44] whereas the relative increase in LBM in treated women did not differ from that in men. The decrease of body fat percentage by 4% and the net 6% increase of lean mass in the male GH-treated group compares favorably with the data of the only published placebo-controlled study for 18 months in men,[13] despite a maintenance GH dose nearly twice as high in our study. At a dose of 9 μg/kg per day and in agreement with studies observing gender differences in GH requirements published after the start of our study,[44–47] the male GH-treated patients and the 9 GH-treated hypogonadal females acquired supraphysiological IGF-I levels whereas at the same GH dose the subgroup of 13 women treated orally with estrogen increased their IGF-I into the desired high normal age-related reference range. Fluid-related adverse events during GH treatment were common (75%), but their early occurrence and even distribution among both genders (despite supraphysiological vs. normal IGF-I levels during treatment in men and women, respectively) indicate that the rate of GH dosing causing rapid IGF-I increase rather than the absolute level of IGF-I obtained was related to the occurrence of side effects. A more gradual increase of the GH dose spanning several months, as is the current practice,[46] titrated to IGF-I levels might have prevented a large number of events.

GH-replacement therapy had a clear effect on all bone parameters studied. Markers of bone formation, OC (+100%) and collagen synthesis, PICP (+50%), increased significantly from the start of GH-replacement therapy onward and this increase, although slowly waning over time, was still significant after 2 years of continuous GH therapy. The bone resorption parameters PYD (+150%) and DPD (+150%) also increased significantly, but this increase waned off during the second year of treatment (Fig. 3). Serum phosphate concentration increased significantly

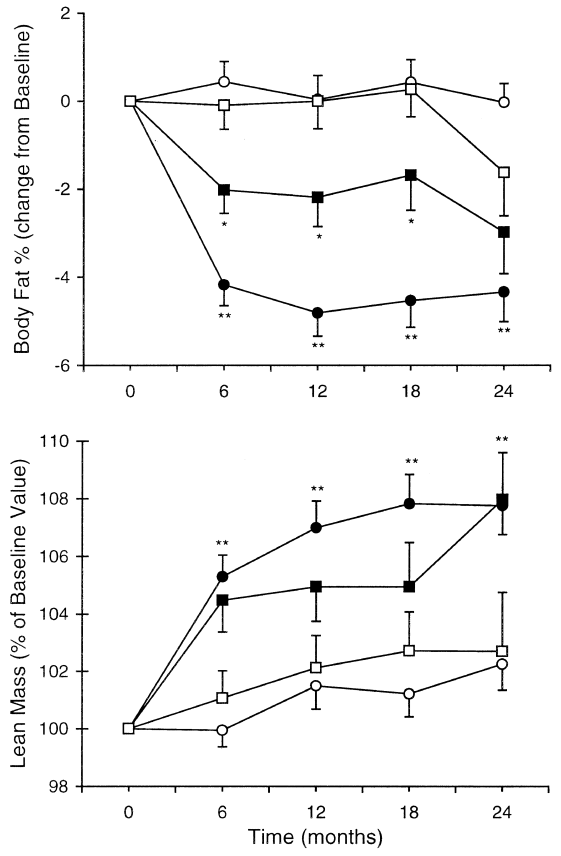

FIG. 5. Change in percentage of body fat (absolute value) and LBM (percentage of baseline) determined by dual-energy X-ray absorption in male (●) and female (■)GH-treated patients and in male (○) and female (□) control patients. Error bars represent ± SEM. Baseline values of percent body fat and LBM differ between genders (Table 2). All values in GH-treated males and females are significantly different from baseline. In addition $**p < 0.001$ for GH-treated males compared with control males. Significant differences in response between GH-treated male and female groups are indicated; $*p < 0.01$.

throughout GH-replacement therapy, reflecting the well-known effect of GH on the tubular reabsorption of phosphate. Serum calcium also slightly but significantly increased (Fig. 3), probably reflecting increased intestinal calcium absorption and accelerated calcium kinetics.[48] All bone parameters increased similarly in GH-treated women and men. The relative extent of accelerated bone turnover as measured by specific biochemical markers corresponds well with histomorphometric data of bone formation and turnover measured in either childhood[49] or adult-onset GHD patients[32] after 1 year of GH-replacement therapy.

Despite a similar increase in bone turnover, only GH-treated males showed a significant increase in bone mass. This positive effect was limited to weight-bearing regions: the lumbar spine (BMC + 7% after 2 years, BMD + 5%; both $p < 0.001$ compared with controls) and the hip (BMD total hip + 3.5%; $p < 0.05$). The increase of 2% at the femoral neck was not significant. At the radius (as measured at two sites), a slight decrease was observed in both genders that was significant versus control group at the UD radius (−2–3% at 2 years of treatment). No significant changes between treated and untreated groups were detectable for total body BMC (Table 3). Cortical bone thickness as measured by quantitative radiogrammetry of the metacarpal was not significantly modified by GH treatment either.

The increase in BMD of the anteroposterior spine in the present controlled study was fairly similar to the effects of GH in adult-onset GHD men studied in the only other (placebo) controlled study reported by Baum.[13] In contrast, they observed no significant changes at the total hip or radius and a 2.4% increase at the femoral neck at 18 months was significant. However, numerous uncontrolled studies (Table 4; see Ohlsson et al.[2]) have revealed a much larger increase of BMC and/or BMD after 2 years or more of GH-replacement therapy. Therefore, it seems likely that prolonged GH-replacement therapy of GHD patients may slowly increase bone mass. The magnitude of such an increase might have been overestimated because of the lack of a suitable control group. Indeed, the GHD control population in the only two controlled long-term studies (Baum[13] and this study) clearly shows that BMC and BMD evolution apparently does not follow the classical expected age-related bone loss. This might be related to a lower bone turnover or to a better compliance to androgen-replacement therapy. A minimum of 6 months of androgen-replacement therapy also may be of too short a duration to allow for complete restoration of the hypogonadal bone loss.[50] On the other hand, the lack of a significant bone loss in the control groups over an 18- to 24-month period confirms previous observations that bone loss in GHD is an early event, independent of the duration of the disease.

Why female patients, despite a more than adequate GH-replacement dose, do not react similarly as GH-treated male patients is unclear. In previous studies lasting 24 months or more and aiming at high normal IGF-I values, women were not included[12,51] or were of low number and not analyzed separately.[8] Some uncontrolled studies mentioned a similar increase in both genders[5,6] or even a small female advantage at the total body.[5] However, more recently, Johansson et al.[10] and Välimäki[11] observed a significant increase in BMC/BMD in GH-treated men but not in women after a follow-up of 33 months and 42 months, respectively. The gender effect observed in our study cannot be ascribed to an insufficient GH dose. Moreover, GH-treated women showed the same relative changes in lean and fat mass, a comparable improvement in QoL, and similar changes in bone turnover markers as GH-treated male patients. Some studies[5,11,52] claimed larger increments in BMD in patients with lower initial Z scores, although this effect has not been confirmed by others.[6,7,10] We did not observe such an effect in our male-treated group; therefore, the higher (normal) initial Z score in our postmenopausal women cannot explain the apparent lack of significant treatment effect in the female group. However, the number of GH-treated women was too small to allow valuable analysis and conclusions regarding the possible confounding effects of age, gonadal status, or (mode of) replacement therapy as modifying factors for their bone response to GH therapy.

Table 4. Controlled and Uncontrolled Studies of at Least 18 Months in Patients With GHD

Reference	No. of patients (No. at final evaluation[b])	Duration (months)	Study design	Percent change in BMD	
				Lumbar spine	Femoral neck
Baum et al.[13]	32 M	18	SBPC	5.1	2.4
Bex et al.	59 M	24	randomized,	5.1	NS
	41 F		open controlled	NS	NS
Johansson et al.[5]	24 M + 20 F	24	Open	3.8	4.1
Janssen et al.[6]	20 M + 27 F	24	Open	2.1	
Johansson et al.[10]	20 M + 13 F (18)	33	Open	1.6[c]	3.5[c]
Rahim et al.[7]	4 M + 3 F	36	Open	3.7	NS
Välimäki et al.[11][a]	44 M + 27 F (32)	36	Open	5.0	5.9
	(20)	42		4.8	5.7
Kann et al.[8]	11 M + 9 F	48	Open	11.8[d]	
Johannsson et al.[9]	31 M + 21 F	48	Open	5.5	7.2
Vandeweghe et al.[4]	20 M CHO (6)	30	Open	5	
ter Maaten et al.[12]	38 M CHO (13)	60	Open	9.6	11.1

M, male; F, female; SBPC, single-blind placebo-controlled; CHO, childhood onset.
[a] Including 12 patients with CHO.
[b] If <50% of included patients evaluated at indicated study duration.
[c] Change versus month 15 of GH replacement.
[d] Nonstable equipment; change in normal controls of 4% at 48 months.

The major limitation of this study is the use of a predetermined GH dose titrated to adverse events and not to measured IGF levels. In retrospect, male and hypogonadal female patients were treated with higher GH doses than recommended nowadays to avoid IGF-I levels above the age-related normal range.[46] Therefore, the results on bone might not be applicable to the current practice. However, despite the supraphysiological dosing, the increase in bone mass is comparable with the results of the only single-blind placebo-controlled study or even less than in numerous open studies (Table 4), using smaller GH doses aiming at normal IGF-I. Furthermore, although randomized and controlled, the study was not placebo-controlled. However, the obvious changes in body composition and the occurrence of side effects would soon have revealed treatment assignment to most patients (and physicians, in case of a double-blind protocol). Finally, because of the unequal inclusion of female versus male patients (enforced by the "no pregnancy" exclusion criterion) the female subgroup analysis might not have had sufficient power to prove a treatment effect.

In conclusion, two controlled studies have now shown a significant positive effect on bone mass and density over an 18- to 24-month GH treatment period in men but no controlled study has yet shown a similar effect in GHD women. However, the true GH effect on bone is underestimated because GH therapy clearly stimulates bone remodeling and thus increases the bone remodeling space (the total volume of osteoclastic resorption pits present at any given time) by ~2%. If bone formation would simply remain in perfect balance with the increased bone resorption during prolonged treatment, BMC and BMD should decrease by ~2% shortly after introduction of GH-replacement therapy and remain stable at that low lower level as long as GH therapy is given. Because BMC/BMD remains about unchanged (in women) and increases significantly (in men), bone balance must be positive during prolonged (>12 months) GH-replacement therapy. The further increase in BMC/BMD observed several months after stopping GH-replacement therapy in Baum's patients,[51] probably because of refilling of the extra remodeling space, is in line with this hypothesis.

The 2-year increase in BMC/BMD in bone areas most at risk for fracture is at first sight lower than what can be obtained by classical antiresorptive agents (estrogens, CT, or bisphosphonates). However, taking into account that the latter agents decrease the remodeling space and GH-replacement therapy increases the remodeling space, the net effect on adult bone balance probably is very similar. Longer-term (>2 year) yet uncontrolled studies suggest indeed that continuous GH therapy further increases BMD by ~2%/year. This increase is not substantially lower than the yearly BMD increment observed in estrogen, SERM, CT, or even bisphosphonate- treated postmenopausal women.[35]

Thus, prolonged GH-replacement therapy in adult-onset GHD patients has a positive effect on bone balance and not only maintains (women) but even increases (men) bone mass, despite a significant increase in bone remodeling.

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