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Effect of Long-Term Growth-Hormone Substitution Therapy on Bone Mineral Density and Parameters of Bone Metabolism in Adult Patients with Growth Hormone Deficiency

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Abstract. Reduced bone mineral density (BMD) and the prevalence for osteoporotic vertebral fractures are symptoms of growth hormone deficiency (GHD) syndrome, and GH replacement therapy is now available for GH-deficient adults. We investigated the long-term effects of GH replacement therapy on bone mineral density (BMD) and bone metabolism in 19 adult patients with GHD over a period of 18 months. In response to GH treatment, the initially decreased IGF-I concentrations rose significantly during 18 months of therapy to levels within the normal range (matched for sex and age) (mean change 158.1 ± 50.8 ng/ml, $P < 0.001$). Parameters of bone formation such as osteocalcin (OC) and procollagen I-C-Peptide (PICP) showed a significant increase in the first 6 months of therapy, followed by a slight decrease in the next months. Markers of bone resorption (Crosslaps^R and deoxypyridinoline (D-Pyr) also increased significantly with a peak value after 6 months and all parameters except PICP remained above baseline values after 18 months. BMD of the femoral neck (FN) showed an increase after 18 months of therapy (mean change 0.01 ± 0.03 g/cm^2 after 18 months, n.s.). However, the increase in BMD was significant only in the lumbar spine (LS) (mean change 0.03 ± 0.04 g/cm^2, $P < 0.05$ after 18 months). We conclude that GH replacement therapy in adult patients with GHD over a period of 18 months causes a pronounced increase in bone turnover mainly during the first 12 months of therapy and increases BMD of the lumbar spine and the femoral neck after 18 months.

Key words: Growth hormone deficiency — GH substitution therapy — Bone mineral density — Bone metabolism — Osteoporosis.

Until recently, the importance of growth hormone deficiency (GHD) in adult patients has not been recognized and GHD adults have not been treated with GH so far. The GHD syndrome in adults is characterized by decreased lean body mass, decreased life expectancy, impaired emotional stress, and reduced bone mineral density (BMD). Therefore, GH replacement therapy has been instituted with increasing frequency.

The role of GH and insulin-like growth factor-I (IGF-I) in bone metabolism has been investigated during the last years *in vitro* and in clinical studies performed mainly for the treatment of osteoporosis [1, 2]. It is well established that GH is important for mature height in several species [3]. It can stimulate chondrocyte growth and function, and thereby the growth of long bones in rodents [3–5]. GH either directly or indirectly via IGF-I increases bone turnover by stimulating osteoblast and osteoclast recruitment and function and inducing collagen synthesis [6]. However, there is some controversy over bone mineral density (BMD) in patients with chronically elevated GH concentrations, i.e., acromegaly. Our group recently reported that chronically elevated GH concentrations cause an increased bone turnover and that BMD is significantly enhanced in the proximal femur but not in the lumbar spine in patients with acromegaly [7]. In contrast, Diamond et al. [8] found a decreased BMD of the lumbar spine which might be related to the hypogonadism often found in patients with acromegaly. In children, GHD results in retarded skeletal growth and GH-replacement therapy has been considered to be an established therapy for many years [9, 10]. GHD adults show a higher incidence of vertebral osteoporotic fractures [11] and have a reduced BMD [12, 13]. It seems that the age of the patient at onset of GHD rather than its duration is the relevant factor for severity of bone loss [12]. Previous studies concerning GH treatment in GHD showed that biochemical markers of bone formation and resorption are significantly elevated in the first 12 months and that BMD decreases after 6 months of therapy but returns to pretreatment values after 12 months [14, 15]. However, the effect of long-term administration of GH on BMD of the femoral neck and lumbar spine is not well defined yet.

The aim of the present study was to investigate the effects of long-term GH-replacement therapy on BMD of the lumbar spine and femoral neck as well as the changes in relevant biochemical parameters of bone metabolism in GH deficient adults.

Subjects and Methods

Patients

Nineteen patients (15 females, 4 males) with a mean age of 45 ±

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Table 1. Patient characteristics

Patient	Sex	Age (Years)	Diagnosis	Other pituitary dysfunction	Hormone replacement therapy
LH	f	48	Endocrine inactive pituitary adenoma	Gonadotr, thyreotr, corticotr	l-thyr 0.1 mg, hydrocortisone 10 mg, est/gest
TC	f	55	Endocrine inactive pituitary adenoma	Gonadotr, thyreotr, corticotr	l-thyr 0.1 mg, hydrocortisone 20 mg
HC	f	56	Prolactinoma	Gonadotr, thyreotr, corticotr	l-thyr 0.1 mg, hydrocortisone 20 mg
SC	f	45	Prolactinoma	Gonadotr	Est/gest
BH	m	31	Prolactinoma	Gonadotr, thyreotr, corticotr	l-thyr 0.1 mg, prednisone 5 mg
GP	m	54	Prolactinoma	—	—
LA	m	27	Idiopathic GH deficiency	Gonadotr, corticotr	Hydrocortisone 30 mg, testosterone 250 mg
ZI	f	60	Prolactinoma	Gonadotr	—
KT	f	45	Prolactinoma	Gonadotr	—
KB	f	46	Meningioma	Gonadotr, thyreotr, corticotr, diab insip	l-thyr 0.1 mg, hydrocortisone 30 mg, desmopressin
RB	f	34	Glia-ependym cyst	Gonadotr, thyreotr, corticotr, diab insip	l-thyr 0.1 mg, hydrocortisone 20 mg, desmopressin
HH	m	59	Prolactinoma	Gonadotr, thyreotr, corticotr	l-thyr 0.1 mg, hydrocortisone 20 mg, testo 250 mg
BC	f	42	Prolactinoma	Gonadotr, thyreotr, corticotr	l-thyr 0.1 mg, hydrocortisone 20 mg
SR	f	28	Prolactinoma	Gonadotr, thyreotr	—
AG	f	39	Prolactinoma	Gonadotr	—
HE	f	52	Craniopharyngioma	Gonadotr, thyreotr	l-thyroxine 0.1 mg
KM	f	28	Idiopathic gh deficiency	Thyreotr	l-thyroxine 0.1 mg
CE	f	43	Endocrine-inactive pituitary adenoma	Gonadotr, thyreotr, corticotr	l-thyroxine 0.1 mg, hydrocortisone 25 mg, est/gest
GU	f	49	Prolactinoma	Gonadotr	Est/gest

Gonadotr = gonadotropic; thyreotr = thyreotropic; corticotr = corticotropic; est/gest = estradiol/gestagen; l-thyr = l-thyroxine; diab insip = diabetes insipidus

2.6 (range 27–60 years), all suffering from GHD, entered this study after informed consent was obtained. The protocol was approved by the Human Ethics Committee of the University of Vienna. The duration of GH deficiency had to be at least 2 years and was documented by an arginine-stimulated GH peak of less than 3 μg/liter after 2 hours. Beside their growth hormone deficiency, all except one of the patients had other pituitary deficiencies and received adequate replacement therapy, which remained unchanged throughout the study. Patient characteristics are given in Table 1. Patients were examined and blood was drawn at baseline and at 3, 6, 9, 12, and 18 months after starting GH replacement therapy.

Study Design

The study was prospective, randomized, and double-blind, and placebo-controlled (GH/Placebo) for the first 6 months. Thereafter all patients received recombinant GH (Genotropin^R, Pharmacia, Stockholm, Sweden) and the patients of the placebo group were added to the treatment group. Patients from the placebo group were tested over a period of 24 months after the start of the study, i.e., over a period of 18 months of GH therapy. Placebo was supplied in identical cartridges for reconstitution with 1 ml of water for injection with 3 mg of m-cresol.

Treatment Schedule

The rhGH dose was 0.125 IU/kg/week (40.5 μg/kg/week) during the first 4 weeks and thereafter 0.25 IU/kg/week (81 μg/kg/week). In two of the patients the dose had to be reduced because of adverse events to 0.125 IU/kg/week and in one patient to 0.08 IU/kg/week. The weekly dose was divided into seven daily s.c. injections at bedtime to mimic the physiological diurnal GH variations as close as possible. Irrespective of the body weight the maximum dose did not exceed 4 IU.

Adverse Events

Three patients suffered from myalgias (two in the therapy, one in the placebo group); six from arthralgias (four in the therapy, two in the placebo group); seven patients showed peripheral edema (five in the therapy, two in the placebo group); and one patient in the placebo group suffered from newly developed headache.

Methods

Blood samples were drawn in the morning at 8 a.m. after an overnight fast and 3 days of a low-collagen diet. IGF-I was measured by a radioimmunoassay (RIA) after treatment of serum samples with acid ethanol to precipitate and neutralize IGF-I binding proteins according to the method of Blum et al. [16]. The minimum detectable IGF-I concentration was 20 ng/ml and intra- and interassay coefficients of variation (CV) were 3.1% and 10%, respectively.

Serum Assays

Serum levels of intact PTH (Nichols Institute Diagnostics, San Juan Capistrano, CA), serum osteocalcin (OC) (CIS International, Gif sur Yvette, France), estradiol, testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroxine were measured by commercially available radioimmunoassays. The intra- and interassay CV were 7.5% and 6.8%, respectively, for intact PTH, and 3.8% and 5.2%, respectively, for serum OC. Serum 25-hydroxyvitamin D_3 concentrations were determined using a protein-binding assay after extraction and purification by chromatography with a C18-hydroxy cartridge (Radiochemical Center, Amersham, UK). The intra- and interassay CV were 6.2% and 8.7%, respectively.

Since type III collagen is a major constituent of most connec-

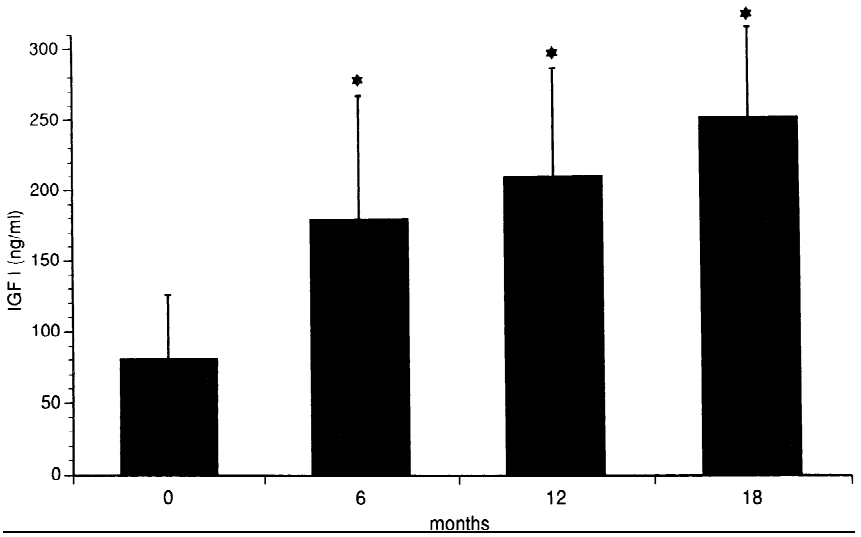

Fig. 1. Time course of IGF-I levels in 19 adult patients with growth hormone deficiency who received human growth hormone (hGH) over a period of 18 months (data are expressed as means ± SD, $*P < 0.05$).

tive tissues in the body but bone matrix contains almost exclusively type I collagen, two different markers of collagen synthesis were assessed to discriminate between general and bone-specific changes in collagen metabolism. The serum concentrations of carboxyterminal propeptide of type I procollagen (PICP) and n-terminal propeptide of type III procollagen (PIIINP) were measured by commercially available RIA (Orion Diagnostica, Espoo, Finland). The intraassay CVs were 2.7% and 2.5%, respectively. The interassay CVs were 6.6% and 3.2%, respectively.

Urinary Assays

CrossLaps^R were measured by a commercially available ELISA (CIS, GIF sur Yvette Cedex, France): The CrossLaps ELISA is based on an immobilized synthetic peptide with an amino acid sequence specific for part of the C-telopeptide of the clarification chain of type I collagen (Glu-Lys-Ala-His-Asp-Gly-Gly-Arg = CrossLaps peptide). The intra- and interassay CV were 2.9% and 5.4%, respectively, and the detection limit was 0.05 μg/ml. Free deoxypyridinoline (D-Pyr) excretion in the urine was measured by commercially available ELISA Pyrilinks-D^R (Metra Biosystems, Inc., Mountain View, CA, USA). Deoxypyrolidine has been shown to be a biochemical indicator of bone resorption and is found only as a degradation product of mature type I collagen of bone and dentin. In the process of bone resorption, D-Pyr is released into the circulation and cleared by the kidney. Pyrilinks-D results are corrected for urinary creatinine concentrations. The intra- and interassay CV were 4.8% and 7.6%, respectively.

Urinary creatinine (Cr) concentrations were measured by standard lab techniques, and the values were used to correct all urinary parameters (CrossLaps^R and D-Pyr).

Bone Densitometry

X-ray films of the lumbar and thoracic spine and the hip were obtained to exclude the possibility of spine fractures or severe osteoarthritis. BMD was measured using dual energy X-ray absorptiometry (DXA) on a QDR 2000™ device (Hologic^R, Waltham, MA, USA). Sites of measurement were the lumbar spine (L1–L4) and the left femoral neck. The *in vivo* precision of DXA was 0.71% for the lumbar spine and 1.0% for the femoral neck. In the first part of the investigation period a second generation densitometer, the Hologic QDR 1000, was used. Cross-calibration during upgrading was performed according to the recommendations made by the manufacturer. Long-term precision was established by performing daily measurements of the hologic spine phantom. The long-term CV was 0.4% (Hologic QDR 1000) and 1% (Hologic QDR 2000), respectively. To reach comparable results during the whole investigation period the first control measurements with QDR 2000 densitometer were scanned with both single-beam and fan-beam modality. These double measurements were made in each of the patients who underwent previous scanning with the QDR 1000.

Statistical Methods

Variables of interest were described by their means and standard deviations (SD). Changes in these variables over time were described by their mean differences and SDs of the differences. Paired *t*-tests, as implemented in the SAS procedure Univariate (SAS, Institute Inc, 1990), were used to test whether the changes in the parameters measured before therapy and a relevant time period after the start of the therapy differ significantly from zero. The relevant time period for IGF-I, femoral neck, and lumbar spine was defined as 18 months; for OC, PICP, PIIINP, Crosslaps, and D-Pyr as 6 months. For IGF-I the changes in the first 6 months were analyzed separately for each treatment group. All *P*-values are based on two-sided tests and the differences were considered significant with $P < 0.05$.

Results

During the first 6 months, the control group receiving placebo showed no changes in IGF-I levels (mean change −3.09 ± 15.79 ng/ml, n.s.). During the same period, IGF-I levels rose significantly in the treatment group, with a mean change of 118.9 ± 99.5 ng/ml, $P < 0.05$ after 6 months and a mean change of 158.2 ± 50.8 ng/ml after 18 months $P < 0.001$) (Fig. 1).

Serum markers of bone formation such as OC and PICP showed a marked and significant increase after 6 months of therapy (OC: mean change 18.9 ± 13.8 ng/ml, $P < 0.001$; PICP: mean change 89.5 ± 66.1 μg/liter, $P < 0.001$) followed by a decrease during the next 12 months. PICP reached pretreatment values after 18 months, but OC and AP remained higher than baseline values. PIIINP as a non-bone-specific marker of collagen production showed similar changes at the various timepoints (Fig. 2).

In accordance with the markers of bone formation, markers of bone resorption such as Crosslaps® and D-Pyr also

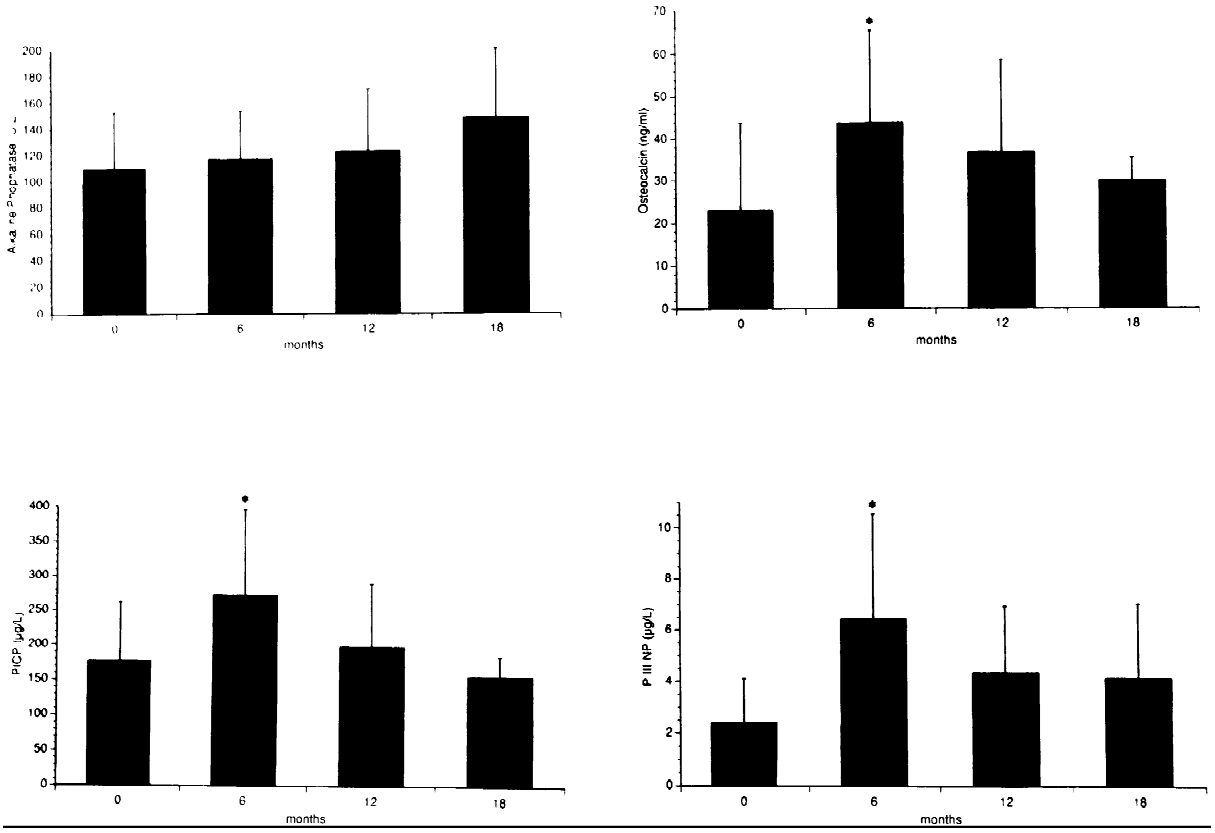

Fig. 2. Time course of **(a)** alkaline phosphatase, **(b)** osteocalcin (OC), **(c)** procollagen-I-C-polypeptide (PICP) and **(d)** propeptide of type III procollagen (PIIINP) in 19 adult patients with growth hormone deficiency who received hGH over a period of 18 months (data are expressed as mean ± SD, *$P < 0.05$).

reached their highest values after 6 months after starting GH therapy (Crosslaps® mean change 136.5 ± 155.9 µg/mmol Cr, $P < 0.05$; D-Pyr mean change 2.4 ± 2.1 nmol/mmol Cr, $P < 0.05$) followed by a slight decrease after 18 months (Fig. 3).

Serum levels of intact PTH and 25(OH)-Vitamin D levels were in the normal range and did not differ significantly during the various timepoints (data not shown).

Before onset of GH substitution therapy in these adult patients with GHD, BMD was decreased 1.0 SD at the femoral neck and 1.3 SD at the lumbar spine when compared with a sex- and age-matched control group (Z-score). BMD of the femoral neck (FN) and the lumbar spine (LS) tended to decrease slightly after 6 months, reached pretreatment values again after 12 months, and then showed a tendency to rise. The increase in BMD of the FN failed to reach statistical significance after 18 months (mean change 0.01 ± 0.03 g/cm^2 after 18 months, n.s.). In contrast, BMD of the LS increased significantly after 18 months of therapy (mean change 0.03 ± 0.04 g/cm^2 after 18 months, $P < 0.05$) (Fig. 4).

Discussion

Our study demonstrates an increase in bone turnover after starting GH replacement therapy in GH-deficient adults. During the first 6 months, we could observe a steady increase in all serum parameters of bone formation (AP, OC, PICP) followed by a slight decrease of OC and PICP in the following 12 months. Parameters of bone resorption (Cross laps®, D-Pyr) increased, with a peak after 6 months, and decreased slightly thereafter but remained above baseline values after 18 months of GH therapy.

In accordance with other studies we saw a decrease of BMD in the FN and LS after 6 months, but BMD of the LS and FN reached pretreatment values after 12 months. After 18 months of therapy BMD of the LS was significantly higher than at baseline. BMD of the FN tended to increase, however, the difference failed to reach statistical significance.

Regulation of bone and calcium metabolism is a complex process involving systemic hormones (PTH, 1,25 $(OH)_2D_3$, calcitonin, sex steroids, thyroxine, and corticosteroids) as well as local growth factors (IGF-I, IGF-II, bFGF, TGF-β) and cytokines (IL-1, IL-3, IL6) [17]. Patients with pituitary insufficiency are often deprived of systemic hormones which can strongly influence bone metabolism, and adequate replacement has to be administered. Bone integrity in adult life is maintained by a narrow coupling of osteoclastic bone resportion and osteoblastic bone formation. Previous human and animal studies have documented GH receptors in rat calvaria and rat epiphyses [18, 19] as well as in cultured human osteoblasts, and it has been shown that IGF-I stimulates bone DNA and collagen synthesis [20] and that GH can increase proliferation of osteoblastic precursor cells [21].

Both IGF-I and IGF-II can stimulate osteoblastic prolif-

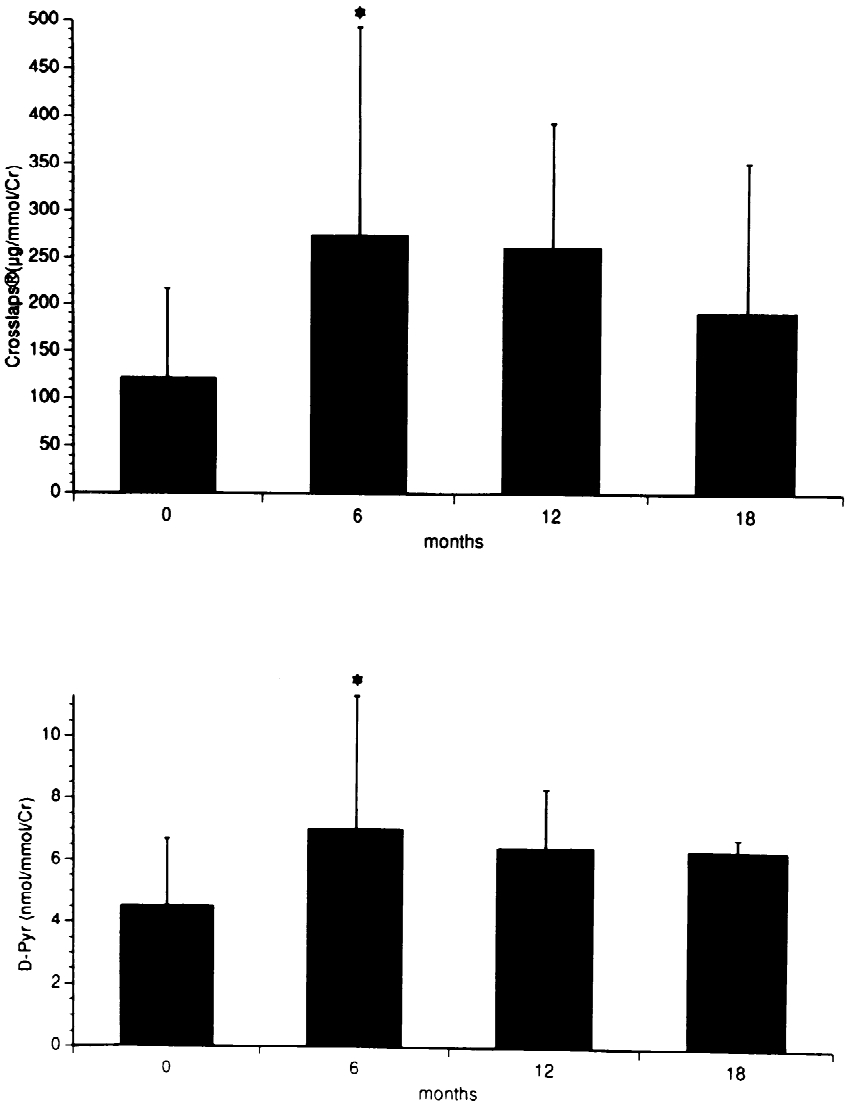

Fig. 3. Time course of **(a)** Crosslaps® and **(b)** deoxypyridinoline (D-Pyr) in 19 adult patients with growth hormone deficiency who received hGH over a period of 18 months (data are expressed as mean ± SD, $*P < 0.05$).

eration and collagen synthesis, however, the role of IGF-I in modulating osteoclast differentiation is still unclear. Hill et al. [22] could show that IGF-I acts indirectly through osteoblastic cells to stimulate osteoclastic activity. On the other hand, Nishiyama et al. [23] were able to demonstrate for the first time a direct effect of GH on osteoclastic bone resorption by direct influence on osteoclast differentiation and indirectly through activation of mature osteoclasts, possibly via stromal cells.

In summary, local production of IGF-I and IGF-II may modulate both osteoblast and osteoclast interactions and functions and play an important role in bone remodeling. In addition to these results, Andreassen et al. [24] demonstrated that hGH injected over 80 days in aged rats induces substantial new subperiostal bone formation and that this new bone showed the same mechanical quality as bone from normal rats. From a subsequent study also dealing with the quality of bone of aged rats they drew the conclusion that mainly cortical and not cancellous bone seems to be affected by the anabolic effect of growth hormone [25].

Adult patients with GHD have significantly lower basal BMD of LS and FN compared with controls [12, 13]. Patients with childhood onset of GHD show especially low BMD levels [9, 10] indicating that possibly reduced bone formation during childhood as well as a negative bone balance in adult GHD patients both contribute to low BMD and consequently lead to the higher incidence of osteoporotic vertebral fractures in these patients [11]. Studies including adults with childhood onset GHD demonstrated a significant increase in vertebral trabecular and cortical bone after 6 and 12 months of GH substitution therapy [26]. Opinions differ on the BMD in mixed populations, including adult and childhood onset GHD: most of them showed decreases in BMD after 3 and 6 months [27] and no significant changes in both cortical and trabecular bone after 12 months, measured at various sites. After 18 months of therapy most of these studies reported a significant increase in cortical and integral bone mass [28–30]. According to these results, Rosen et al. [31] could demonstrate, in a long-term study over 18 months of GH treatment in a population of 12 patients including only adults with adult onset GHD, that BMD at the FN increased during 18 months whereas BMD at the same site did not change after 6 and 12 months. They speculated that it may take at least 18 months of therapy before an increase in mineralized bone could be noticed.

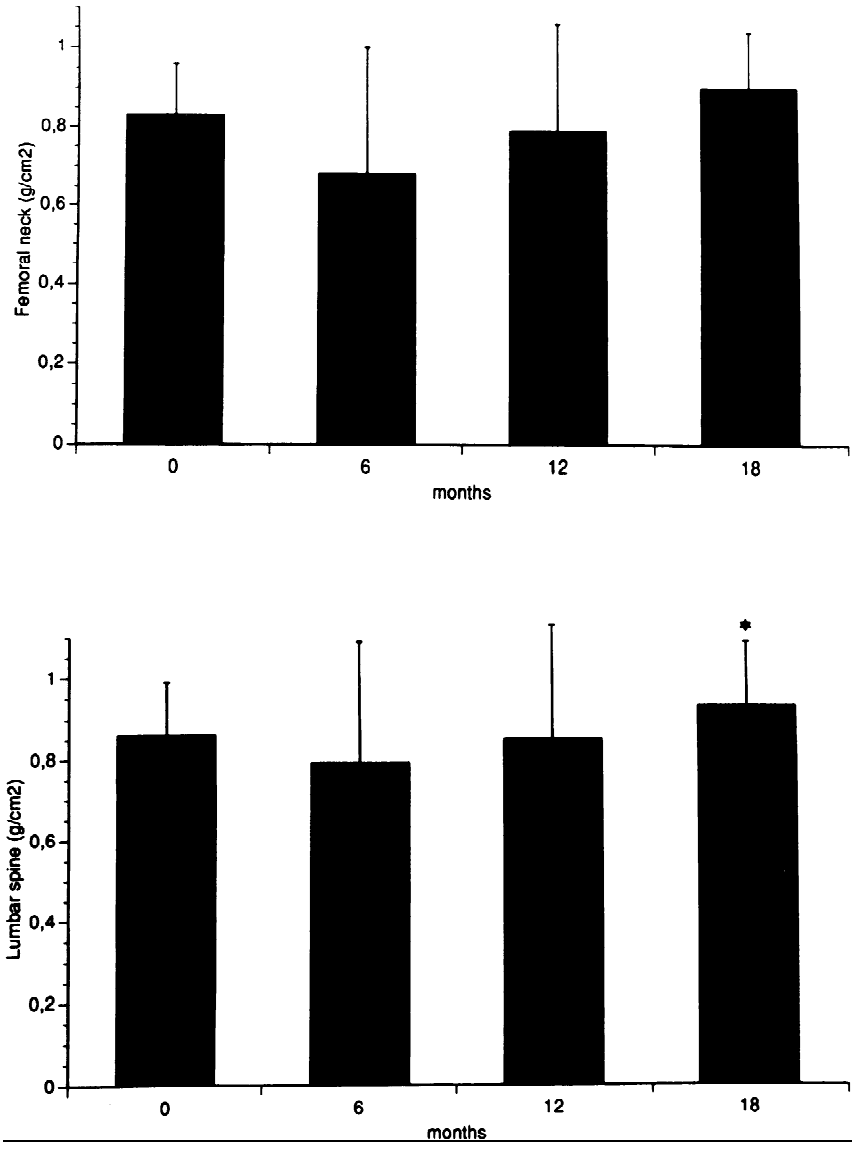

Fig. 4. Time course of bone mineral density of the **(a)** femoral neck and **(b)** lumbar spine in 19 adult patients with growth hormone deficiency who received hGH over a period of 18 months (data are expressed as mean ± SD, $*P < 0.05$).

From our study we conclude that long-term therapy with GH leads to a simultaneous increase in bone formation and bone resorption. In comparison to other studies, it seems that the initially observed increase in bone resorption with a stronger recruitment of mature osteoclasts results in a negative net effect in BMD after 6 months before a new steady state in bone formation and degradation is obtained after 12 months of therapy. Similar to the study of Vandeweghe et al. [29] we found that, although the markers of bone turnover do not increase further after 12 months, there is a positive net effect in BMD after 18 months which is more pronounced in the lumbar spine than in the femoral neck. GH therapy may influence cortical bone differently from trabecular bone, perhaps by a slower increase of bone turnover and lower mineral apposition rates. Trabecular bone appears to be more sensitive to the effects of GH than cortical bone in these patients.

In conclusion, long-term substitution therapy with rhGH in GHD adults causes an enhanced bone turnover and, after 18 months of therapy, a significant increase in BMD at the lumbar spine can be noticed. Thus, it can be expected that long-term therapy with GH, besides other benefits, might decrease the frequency of vertebral fractures and reduce bone fragility in GHD patients over a longer period of time. Long-term studies over 3 and 5 years will be necessary to relate these beneficial effects on BMD and fracture risk.

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