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Calcium Supplementation Increases Bone Mass in GH-Deficient Prepubertal Children during GH Replacement

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Key Words

Growth hormone-deficient children · Calcium supplementation · Bone mineral content · Bone mineral density · Dual-energy X-ray absorptiometry

Abstract

Background/aims: Since GH plays an important role in bone mineralization, and several studies demonstrated the positive influence of a higher calcium intake on bone mass, we studied the effect of calcium supplementation in GHD children during GH therapy. ***Methods:*** 28 prepubertal GHD children, 5.0–9.9 years old, were assigned to two groups: group A (n = 14; 7 females) treated with GH, and group B (n = 14; 7 females) treated with GH + calcium gluconolactate and carbonate (1 g calcium/day per os). Auxological parameters, total bone mineral content (TBMC) and density (TBMD), leg BMC and BMD, lumbar BMD, fat mass (FM) and lean tissue mass (LTM), blood 25-hydroxyvitamin D (25-OHD), parathyroid hormone (PTH), osteocalcin (OC) and urinary N-terminal telopeptide of type I collagen (NTx) were determined at the start of therapy and after 1 and 2 years of treatment. ***Results:*** During the 2 years of the study, TBMC, TBMD, leg BMC and BMD (but not lumbar BMD) increased in both groups of patients, however after 2 years of treatment they were significantly higher in the calcium-supplemented group B than in group A ($p < 0.05$, for all parameters). At the start of therapy, in both groups of patients percentage FM was higher and total and leg LTM lower than in controls ($p < 0.05$ for each parameter). Thereafter, FM decreased and LTM increased and after 2 years they were both different from baseline ($p < 0.05$). After 2 years of treatment, leg BMC and BMD were more positively correlated with regional leg LTM in patients of group B ($r = 0.834$ and $r = 0.827$, respectively; $p < 0.001$) than in patients of group A ($r = 0.617$ and $r = 0.637$, respectively; $p < 0.05$). 25-OHD and PTH levels were in the normal range in all patients at the start and during treatment. OC levels were lower and urinary NTx levels higher in patients than in controls ($p < 0.05$ for both parameters), either at the start and after 1 year of treatment. After 2 years of treatment, OC levels were significantly higher than at the start of the study ($p < 0.05$) in both groups of patients, but they were higher in group B than in group A ($p < 0.05$); on the contrary, urinary Ntx levels were lower in group B than in group A ($p < 0.05$). ***Conclusion:*** In GHD children, treated with GH, calcium supplementation improved bone mass; it may aid in reaching better peak bone mass and in protecting weight-bearing bones, usually completed in childhood to maximum levels, from risk of osteoporosis and fractures later in life.

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Introduction

It is well known that growth hormone-deficient (GHD) children have reduced bone mineral density (BMD), because of delayed bone maturation and absence of GH anabolic actions on bone [1]. GH therapy improves BMD [2], however childhood-onset GHD patients have an increased susceptibility to osteoporosis, with its attendant risks of fragility fracture later in life. In fact, peak bone mass (PBM) occurs several years after the completion of linear growth, in late adolescence and early adulthood, and the discontinuation of GH therapy at the completion of linear growth could limit the attainment of PBM [3, 4]. Therefore, the GHD patients not adequately treated could not reach PBM at the time of discontinuation of GH therapy and could show a bone mineral mass lower than their genetic potential. Consequently, the normalization of bone mass deficit represents a major advance in the prevention of osteoporosis and bone fractures [5]. The variability in PBM is accounted for by genetic factors predominantly; however, even environmental influences, as endocrine function, physical activity and nutrition, may affect the ability of an individual to reach the genetic potential for bone mass [6], as well as interactions between environmental and genetic factors.

Concerning nutrition, randomized controlled studies demonstrated that calcium intake correlated with BMD in healthy adolescents [7, 8], that calcium supplementation above the recommended dietary allowances increased BMD in prepubertal children [9, 10], that PBM was optimal when the threshold calcium balance was met [11] and that calcium supplementation increased the bone mineral content (BMC) of children with habitually low calcium intake [12]. In addition, we showed in previous studies that calcium supplementation improved bone mass in females affected by central precocious puberty and treated with GnRH agonists [13, 14]. On these bases, the aim of the present longitudinal study was to determine whether calcium supplementation improved bone mass also in prepubertal GHD children treated with GH and supplemented with calcium.

Patients and Methods

Patients

Twenty-eight prepubertal Caucasian GHD children (14 females), 5.0–9.9 years old, took part in the study. The diagnosis of GHD was based on the following criteria: height <2 SDS; bone age (BA) delay >2 years compared with chronological age (CA); GH peak <10 ng/ml in at least two consecutive pharmacological tests; insulin-like growth factor I (IGF-I) levels below the mean of age- and sex-matched controls. None of the patients had organic GH deficiency or panhypopituitarism or multiple pituitary hormone deficiency, all being affected by idiopathic isolated GHD, as assessed by full endocrine evaluation and magnetic resonance imaging of the hypothalamic-pituitary and stalk-pituitary region.

Patients, at the start of treatment with recombinant GH at a dose of 0.033 mg/kg 6 days/week, were randomly assigned to two groups (A and B) comparable for sex, CA, BA, weight and height, using a computer pseudorandom number generator. Patients in group A (n = 14; 7 females) were treated solely with GH; patients in group B (n = 14; 7 females) received a treatment with GH plus supplementation of a commercial combination pill of calcium gluconolactate and carbonate (1 g calcium/day per os in two doses).

Height, weight, body mass index (BMI), total bone mineral content (TBMC) and density (TBMD), lumbar spine BMD and leg BMC and BMD, fat percentage (fat %) and total lean mass (kg), plasma 25-hydroxyvitamin D (25-OHD) and serum parathyroid hormone (PTH) levels, serum osteocalcin (OC) as marker of bone formation and urinary N-terminal telopeptide of type I collagen (NTx) as marker of bone resorption, urine calcium/creatinine ratio, were determined at the start of therapy and after 1 and 2 years of treatment. No patients received other drugs known to interfere with bone mineral metabolism. Renal and hepatic functions were normal.

All the subjects were instructed to continue their usual physical activity and diet, thereby ensuring adequate caloric (70–80 cal/kg/day), protein (>1 g/kg/day), calcium (>800 mg/day), and phosphate (>800 mg/day) intake during treatment. Diet was investigated by a weighed food record and dietary calcium intake was determined by a detailed food frequency questionnaire of dairy products [15]. Compliance in assumption of calcium supplementation was checked by a diary in patients of group B. Physical activity, including physical education classes, organized sports, recreational activity, and habitual walking and cycling, was investigated by an exercise diary and was measured in minutes per week [16].

At the start and after 1 and 2 years of treatment, auxological, body composition, and bone densitometric data of patients were compared with those of different control groups of the same CA, with BA appropriate for CA, and BMI between the 25th and 75th percentile [17]; they were composed of 26 (13 females), 24 (12 females) and 28 (14 females) children, at the start of the study and after 1 and 2 years, respectively. The inclusion criteria, at each time point of the study, were: age-matching with the patient population, white race, prepubertal stage and normal calcium intake. The exclusion criteria were: a history of metabolic bone, kidney, liver, gastric and bowel disease; other systemic chronic disease, and use of medications known to affect bone metabolism.

Informed consent was obtained from all parents of study participants and the study protocol was approved by our institutional ethics committee.

Methods

Weight was measured with minimal clothing and without shoes on a standard clinical balance. Standing height was measured by a Harpenden stadiometer. BMI was calculated as weight (kg)/height (m^2) and compared with age- and sex-matched reference values [17] to calculate SD scores (SDs). BA evaluation was determined blindly by the same expert observer according to the method of Greulich and Pyle [18] and expressed in years.

Plasma 25-OHD was measured by high-pressure liquid chromatography (HPLC) (Eureka srl, Chiaravalle, Ancona, Italy). The intra- and interassay coefficients of variations (CVs) were below 5.2 and 7.8%, respectively. The sensitivity was 5 nmol/l. Serum intact 1-84 PTH levels were determined by immunometric chemiluminescence assay (Nichols Institute Diagnostics, San Clemente, Calif., USA). The intra- and interassay CVs were below 6.7 and 9.2%, respectively. The sensitivity was 0.1 pmol/l. Serum OC was measured by immunometric chemiluminescence assay (Diagnostic Products Corp., Los Angeles, Calif., USA). The intra- and interassay CVs were below 4.5 and 7.1%, respectively. The sensitivity was 0.02 nmol/l. Urine levels of NTx were measured by an enzyme immunosorbent assay (Osteomark; Ostex, Seattle, Wash., USA). Urine specimens were collected between 10:00 and 12:00 h as the second voiding of the day. Assay values were expressed in nanomoles bone collagen equivalents per liter (nmol BCE/l). The sample results from a single urine collection were normalized for urine dilution by urine creatinine analysis and were reported as nmol BCE/mmol creatinine. The intra- and interassay CVs were less than 9%. The sensitivity was 20 nmol BCE/l. Urine calcium and creatinine were analyzed using standard laboratory methods and were expressed as urine Ca/Cr ratio (μmol/mmol).

TBMC (g), TBMD (g/m^2), leg BMC and BMD, lumbar spine BMD, fat mass (FM) and lean tissue mass (LTM) were measured using dual-energy X-ray absorptiometry (DXA equipped with pediatric software; Lunar Corp., Madison, Wisc., USA), as previously reported [14, 19], and assessed by the same blinded reader. The second, third, and fourth lumbar vertebrae were scanned by anteroposterior projection (AP-BMD). Only the third lumbar vertebra was also measured by lateral scan (L-BMD) because of possible interference with the assessment of the second and fourth lumbar vertebrae by overlying ribs or iliac crests, respectively. True volumetric BMD (vBMD) was calculated, expressed in g/cm^3, taking the vertebral body as an ellipsoid cylinder and dividing BMC obtained by lateral scan (g) by the body vertebral volume (cm^3), calculated as: $\Delta \times \text{width}/2 \times \text{depth} \times \text{height}$, to reduce the confounding effect of bone size [20]. Vertebral dimensions (anterior width, depth, and height) were obtained using software data. DXA was calibrated daily using a commercial phantom to exclude measurements drifts during the study period. The CVs for duplicate measurements in normal children at an interval of 1 week were 1% for AP-BMD, 1.2% *L*-BMD, and 2.8% vBMD. The CVs were 0.6% for TBMC, 1.0% for TBMD, 1.2% for leg BMC, 1.3 for leg BMD and 2.2% for FM and LTM.

Height, weight and BMI standard deviation scores (SDs) were calculated as: (measured value – mean population value)/SD of the normal population.

TBMC, TBMD, leg BMC and BMD and lumbar spine BMD were correlated with FM and total and leg LTM by multiple regression analysis controlling for age and sex, using bone parameters as the dependent variables.

Statistical Analysis

Descriptive analyses are expressed as mean ± SD. Statistical analyses were performed using unpaired t test, ANOVA, and multiple regression analysis. All statistical analyses were performed using a data analysis system (StatView 4.5; Abacus Concepts, Inc., Berkeley, Calif., USA) run on an Apple PowerMac Computer (Apple Computer, Inc., Cupertino, Calif., USA). Statistical significance was set at $p < 0.05$.

Results

Table 1 shows clinical data of the two groups of patients at the start of the study and after 1 and 2 years of treatment. The two groups of patients were comparable for sex, CA, BA, height, height velocity, weight and BMI, at the start and after 1 and 2 years of treatment. The patients studied were all prepubertal even after 2 years of treatment. At the start of treatment, BA, height and height velocity were significantly lower in the two groups of patients than in controls ($p < 0.05$). In both groups of patients, height and height velocity rate significantly increased after 1 and 2 years of treatment in comparison to pretreatment values ($p < 0.05$ for both parameters and for both groups). In both groups of patients, BMI was slightly higher at the start of the study than in controls and slightly decreased after 2 years of treatment, but the differences were not statistically significant.

There were no differences in physical activity or exposure to sunlight between the two groups of patients, as reported in each patient's exercise diary. Only during the second year of treatment was the physical activity slightly higher in boys than in girls (mean 8.1 vs. 7.4 h/week), but the difference was not statistically significant. Concerning calcium intake, at the start of treatment it was comparable in the two groups of patients (887 ± 54 mg/day in group A and 864 ± 39 mg/day in group B) and in controls (855 ± 43 mg/day). Thereafter, calcium intake was comparable only in patients of group A (895 ± 48 mg/day after 1 year; 901 ± 36 mg/day after 2 years of treatment) and in controls of the same CA (870 ± 37 and 888 ± 57 mg/day, respectively). The compliance of calcium supplementation in patients of group B was more than 91% in the first year and more than 88% in the second year. In fact, calcium intake in group B was 1,645 ± 152 mg/day during the first year of calcium supplementation and 1,562 ± 171 mg/day during the second year, as reported in each patient's food diary.

Table 2 shows densitometric data in the two groups of patients studied, at the start of therapy and after 1 and 2 years of treatment. At the start of treatment, TBMC, TBMD, leg BMC and BMD, and lumbar BMD were significantly lower in both groups of patients than in controls ($p < 0.05$ for all parameters). During the 2 years of the study, TBMC, TBMD, and leg BMC and BMD increased in patients of both groups and in controls of the same CA. After 2 years of treatment, TBMC, TBMD and leg BMC and BMD were found to be significantly higher in the calcium-supplemented group B than in the unsupple-

Table 1. Auxological data of the patients studied at the start and after 1 and 2 years of treatment

	Start			1 year			2 years		
	controls (n = 26)	group A	group B	controls (n = 24)	group A	group B	controls (n = 28)	group A	group B
CA, years	8.30 ± 1.22	8.25 ± 1.50 (5.0–9.9)	8.21 ± 1.41 (5.1–9.8)	9.25 ± 1.14	9.22 ± 1.51 (6.0–10.9)	9.21 ± 1.41 (6.1–10.8)	10.33 ± 1.08	10.24 ± 1.49 (7.1–11.9)	10.23 ± 1.42 (7.1–11.7)
BA, years	8.4 ± 1.2	6.3 ± 1.4[a]	6.2 ± 1.3[a]	9.2 ± 1.3	7.3 ± 1.4[a]	7.4 ± 1.4[a]	10.3 ± 1.4	8.6 ± 1.3[a]	8.7 ± 1.2[a]
Height, cm	128.5 ± 6.5	113.0 ± 7.9[a]	112.8 ± 6.6[a]	134.2 ± 6.1	120.6 ± 7.8[b]	121.2 ± 6.6[b]	139.4 ± 6.3	128.0 ± 8.1[b]	128.3 ± 6.8[b]
Height, SDs	0.31 ± 0.37	–2.51 ± 0.34[a]	–2.53 ± 0.36[a]	0.23 ± 0.28	–1.98 ± 0.32[b]	–1.95 ± 0.29[b]	0.33 ± 5.72	–1.49 ± 0.25[b]	–1.46 ± 0.17[b]
Δ height SDs/years					0.54 ± 0.27	0.59 ± 0.30		0.50 ± 0.32	0.52 ± 0.28
Height velocity, cm/year	6.55 ± 1.26*	3.66 ± 0.50*, [a]	3.62 ± 0.52*, [a]	5.83 ± 1.80*	7.73 ± 0.73[b]	8.16 ± 1.01[b]	5.68 ± 1.92*	7.36 ± 1.11[b]	7.20 ± 1.09[b]
Weight, kg	27.5 ± 2.6	22.4 ± 2.8	22.6 ± 3.0	30.2 ± 2.9	25.1 ± 3.15	25.6 ± 3.19	32.8 ± 3.42	28.1 ± 3.87	28.3 ± 4.07
Weight, SDs	0.12 ± 0.48	–1.14 ± 0.46	–1.06 ± 0.54	0.05 ± 0.38	–0.98 ± 0.44	–0.89 ± 0.46	0.33 ± 0.35	–0.58 ± 0.43	–0.55 ± 0.42
BMI, kg/m^2	16.7 ± 0.8	17.5 ± 1.0	17.8 ± 1.2	16.8 ± 0.79	17.3 ± 1.03	17.5 ± 1.17	16.9 ± 0.98	17.1 ± 1.12	17.2 ± 1.11
BMI, SDs	0.25 ± 0.13	0.42 ± 0.12	0.46 ± 0.14	0.28 ± 0.18	0.40 ± 0.15	0.51 ± 0.13	0.20 ± 0.19	0.23 ± 0.18	0.25 ± 0.22

Group A: patients treated solely with hGH. Group B: patients treated with hGH and supplemented with calcium. All data are expressed as mean ± SD. CA = Chronological age; BA = bone age; BMI = body mass index.

* During the previous 12 months; [a] vs. controls: $p < 0.05$; [b] vs. pretreatment: $p < 0.05$.

Table 2. Densitometric data of the patients studied, at the start and after 1 and 2 years of treatment

	Start			1 year			2 years		
	controls	group A	group B	controls	group A	group B	controls	group A	group B
TBMC, g	889.0 ± 78.5	827.5 ± 135.0[c]	832.7 ± 123.2[c]	924.4 ± 83.3	876.1 ± 141.2[c] (5.9)	901.2 ± 150.6 (8.1)	971.8 ± 87.0	908.9 ± 147.3 (9.9)	947.2 ± 162.2[a] (13.7)
TBMC, SDs		–0.79 ± 0.57	–0.73 ± 0.54		–0.58 ± 0.62	–0.28 ± 0.55		–0.72 ± 0.61	–0.28 ± 0.56
TBMD, g/cm^2	0.846 ± 0.39	0.810 ± 0.033[c]	0.815 ± 0.027[c]	0.868 ± 0.048	0.832 ± 0.039 (2.7)	0.849 ± 0.032 (4.1)	0.889 ± 0.050	0.857 ± 0.037 (5.8)	0.886 ± 0.035[a] (8.7)
TBMD, SDs		–0.92 ± 0.56	–0.79 ± 0.48		–0.75 ± 0.61	–0.40 ± 0.48		–0.64 ± 0.52	–0.06 ± 0.45
Leg BMC, g	266.7 ± 22.1	243.1 ± 26.4[c]	245.6 ± 24.5[c]	279.3 ± 28.3	254.0 ± 27.5[c] (4.5)	267.9 ± 33.6 (9.4)	294.6 ± 33.2	268.8 ± 34.5 (10.7)	288.2 ± 21.5[a] (17.5)
Leg BMC, SDs		–1.05 ± 0.44	–0.95 ± 0.53		–0.89 ± 0.62	–0.39 ± 0.59		–0.76 ± 0.50	–0.18 ± 0.49
Leg BMD, g/cm^2	0.861 ± 0.034	0.795 ± 0.033[c]	0.800 ± 0.027[c]	0.878 ± 0.042	0.823 ± 0.038 (3.5)	0.845 ± 0.032 (5.6)	0.895 ± 0.044	0.851 ± 0.036 (7.0)	0.898 ± 0.034[a] (12.2)
Leg BMD, SDs		–1.94 ± 1.02	–1.79 ± 1.11		–1.31 ± 0.98	–0.79 ± 1.05		–1.00 ± 0.79	0.07 ± 0.80
Lumbar BMD, g/cm^2	0.720 ± 0.038	0.677 ± 0.048[c]	0.673 ± 0.075[c]	0.750 ± 0.040	0.722 ± 0.053 (6.6)	0.726 ± 0.075 (7.9)	0.785 ± 0.052	0.764 ± 0.064 (12.8)	0.787 ± 0.090 (16.9)
Lumbar BMD, SDs		–1.13 ± 0.67	–1.24 ± 0.70		–0.70 ± 0.65	–0.60 ± 0.68		–0.50 ± 0.79	0.05 ± 0.85
vBMD L3, g/cm^3	0.220 ± 0.029	0.204 ± 0.042	0.202 ± 0.034	0.235 ± 0.031	0.217 ± 0.043 (6.3)	0.219 ± 0.032 (8.4)	0.249 ± 0.034	0.232 ± 0.041 (13.7)	0.240 ± 0.031 (18.8)
vBMD L3, SDs		–0.55 ± 0.38	–0.62 ± 0.40		–0.58 ± 0.43	–0.52 ± 0.66		–0.50 ± 0.55	–0.26 ± 0.50
FM, %	15.4 ± 5.7	20.7 ± 6.0[c]	19.7 ± 5.3[c]	16.0 ± 4.9	17.9 ± 7.0 (–13.5)	17.2 ± 6.4 (–12.7)	15.8 ± 4.7	16.7 ± 5.2[b] (–19.3)	16.3 ± 4.6[b] (–17.3)
FM, % (SDs)		0.93 ± 0.60	0.75 ± 0.57		0.39 ± 0.49	0.24 ± 0.51		0.19 ± 0.44	0.11 ± 0.47
FM, kg	4.2 ± 0.8	4.6 ± 0.9	4.5 ± 0.7	4.8 ± 0.9	4.5 ± 0.6 (–2.2)	4.4 ± 0.5 (–2.2)	5.2 ± 1.0	4.8 ± 0.8 (4.3)	4.5 ± 0.7 (0.0)
FM, kg (SDs)		0.50 ± 0.30	0.38 ± 0.33		–0.33 ± 0.29	–0.44 ± 0.30		–0.40 ± 0.28	–0.70 ± 0.39
Total LTM, %	81.4 ± 4.2	75.6 ± 3.9[c]	76.6 ± 4.4[c]	80.9 ± 4.7	78.6 ± 3.6 (3.9)	79.3 ± 4.1 (3.5)	81.2 ± 4.0	79.9 ± 3.8[b] (5.7)	80.5 ± 4.2[b] (5.1)
Total LTM, % (SDs)		–1.38 ± 0.69	–1.14 ± 0.75		–0.49 ± 0.62	–0.34 ± 0.70		–0.32 ± 0.55	–0.18 ± 0.52
Total LTM, kg	22.4 ± 1.8	16.9 ± 1.5[c]	17.1 ± 1.6[c]	24.4 ± 1.9	19.7 ± 1.9[c] (16.6)	20.3 ± 1.7[c] (18.7)	26.7 ± 2.1	22.3 ± 1.8[b] (31.9)	22.9 ± 1.9[b] (33.9)
Total LTM, kg (SDs)		–3.06 ± 1.18	–2.94 ± 1.09		–2.47 ± 1.11	–2.16 ± 1.20		–2.10 ± 0.79	–1.81 ± 0.70
Leg LTM, kg	7.1 ± 1.6	5.1 ± 1.0[c]	5.2 ± 0.9[c]	7.7 ± 1.9	5.9 ± 1.2[c] (15.7)	6.1 ± 1.1[c] (17.3)	8.5 ± 1.8	6.7 ± 1.2[b] (31.4)	7.0 ± 1.2[b] (34.6)
Leg LTM, kg (SDs)		–1.25 ± 0.98	–1.19 ± 0.85		–0.95 ± 0.71	–0.84 ± 0.73		–1.00 ± 0.56	–0.83 ± 0.69

Group A: patients treated solely with hGH. Group B: patients treated with hGH and supplemented with calcium. All data are expressed as mean ± SD. Percentage variation versus baseline (Δ%) in parentheses.

TBMC = Total bone mineral content; TBMD = total bone mineral density; FM = fat mass; LTM = lean tissue mass. [a] $p < 0.05$ vs. group A; [b] $p < 0.05$ vs. baseline; [c] $p < 0.05$ vs. controls. The percentage variation (Δ%) in the measured parameters was calculated as: [(measured value – initial value)/initial value] × 100.

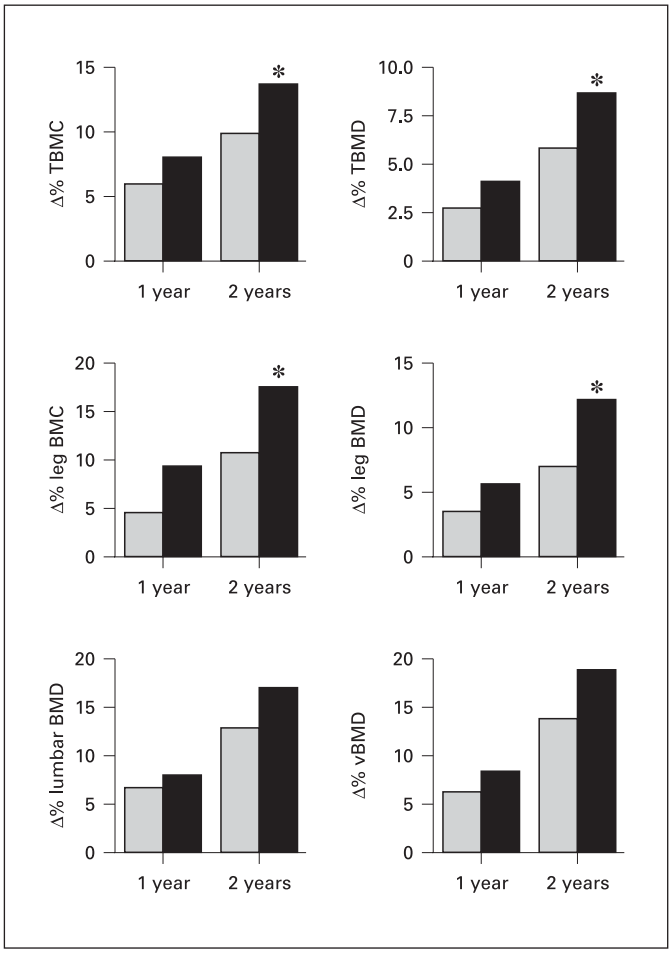

Fig. 1. Percentage variation versus baseline (Δ%) for densitometric data of the patients studied, after 1 and 2 years of treatment. Group A: patients treated solely with hGH (grey columns); group B: patients treated with hGH and supplemented with calcium (black columns). * $p < 0.05$ vs. group A.

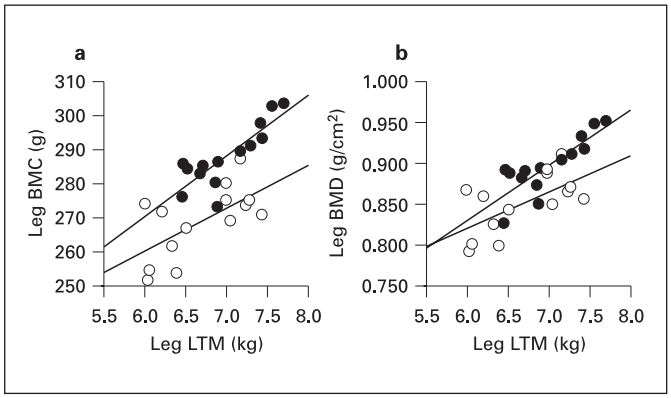

Fig. 2. Correlation between leg BMC and BMD and regional leg tissue mass (LTM) in patients treated solely with hGH (group A – white circles) and in patients treated with hGH and supplemented with calcium (group B – black circles), after 2 years of treatment. Regression analysis shows leg BMC (1) and BMD (2) more positively correlated with regional leg LTM in patients of group B than in patients of group A. **a** Group A: $r = 0.617$; $p < 0.05$. Group B: $r = 0.834$; $p < 0.01$. **b** Group A: $r = 0.637$; $p < 0.05$. Group B: $r = 0.827$; $p < 0.01$.

mented group A ($p < 0.05$, for all these parameters). Lumbar BMD and vBMD of the third lumbar vertebra slightly increased, and were not significantly different in the two groups of patients. Figure 1 shows percentage variation versus baseline for densitometric data of the patients studied, at the start and after 1 and 2 years of treatment.

At the start of therapy, in both groups of patients percentage body FM was significantly higher and total and leg LTM significantly lower than in controls ($p < 0.05$ for each parameter). Thereafter, FM decreased and LTM increased and after 2 years of treatment their difference versus baseline values was statistically significant ($p < 0.05$ for both parameters). After 1 year of therapy, TBMC, TBMD and leg BMC and BMD were positively correlated with LTM in both groups of patients ($p < 0.05$ for each bone parameter). After 2 years of treatment (fig. 2), leg BMC and BMD were more positively correlated with regional leg LTM in patients of group B ($r = 0.834$ and $r = 0.827$, respectively; $p < 0.001$) than in patients of group A ($r = 0.617$ and $r = 0.637$, respectively; $p < 0.05$).

Table 3 shows bone marker data of the patients studied, at the start and after 1 and 2 years of treatment. Blood 25-OHD and PTH levels were in the normal range and comparable in the two groups of patients and in controls at the start of the study and after 1 and 2 years. Creatinine excretion was not modified by GH treatment. Urine Ca/Cr ratio was comparable in the two groups of patients and in controls at the start of the study and after treatment. Serum OC levels were significantly lower and urinary NTx levels significantly higher in patients than in controls ($p < 0.05$ for both parameters, either at the start or after 1 year of treatment). After 2 years of treatment, OC levels were significantly higher than at the start of the study ($p < 0.05$) in both groups of patients; but they were significantly higher in group B than in group A ($p < 0.05$); on the contrary, urinary NTx levels were significantly lower in group B than in group A ($p < 0.05$).

Table 3. Bone marker data of the patients studied, at the start and after 1 and 2 years of treatment

	Start			1 year			2 years		
	controls	group A	group B	controls	group A	group B	controls	group A	group B
25-OHD, nmol/l	58 ± 13	62 ± 15	57 ± 12	61 ± 17	64 ± 19 (+3.2)	59 ± 15 (–3.5)	49 ± 13	60 ± 18 (–3.2)	56 ± 14 (–1.7)
25-OHD, SDs		0.31 ± 0.95	–0.08 ± 0.89		0.17 ± 0.78	–0.12 ± 0.87		0.85 ± 0.94	0.54 ± 0.76
PTH, pmol/l	2.8 ± 0.7	2.6 ± 0.6	2.9 ± 0.8	3.1 ± 1.1	2.9 ± 1.0 (+11.5)	2.6 ± 0.9 (–10.3)	3.4 ± 1.2	3.1 ± 1.0 (+18.2)	2.7 ± 1.1 (–6.9)
PTH, SDs		–0.29 ± 0.58	0.14 ± 0.65		–0.18 ± 0.70	–0.45 ± 0.80		–0.25 ± 0.77	–0.58 ± 0.93
Oc, nmol/l	3.13 ± 0.72	1.41 ± 0.33[c]	1.58 ± 0.43[c]	3.49 ± 0.82	2.56 ± 0.40[b] (+81.5)	2.68 ± 0.48[b] (+69.6)	3.69 ± 0.68	2.47 ± 0.41[b] (+75.2)	3.01 ± 0.55[a, b] (+90.5)
Oc, SDs		–2.39 ± 1.13	–2.15 ± 1.31		–1.13 ± 1.03	–0.99 ± 1.02		–1.79 ± 0.78	–1.00 ± 0.67
uNTx, nmol BCE/ mmol creatinine	345 ± 110	638 ± 145[c]	581 ± 156[c]	386 ± 123	561 ± 167[c] (–12.7)	534 ± 145[c] (–8.1)	378 ± 125	702 ± 174[c] (+10.3)	530 ± 132[a, c] (–8.8)
uNTx, SDs		2.66 ± 1.20	2.16 ± 1.18		1.42 ± 0.58	1.20 ± 0.49		2.59 ± 1.14	1.22 ± 0.82
uCa/Cr, µmol/mmol	242 ± 78	244 ± 82	242 ± 73	240 ± 85	243 ± 88 (–0.4)	241 ± 75 (–0.4)	237 ± 90	243 ± 79 (–0.4)	238 ± 90 (–1.24)
uCa/Cr, SDs		0.03 ± 0.88	–0.00 ± 0.76		0.04 ± 0.83	0.01 ± 0.90		0.07 ± 0.81	0.01 ± 0.79

Group A: patients treated solely with hGH. Group B: patients treated with hGH and supplemented with calcium. All data are expressed as mean ± SD. Percentage variation versus baseline (Δ%) in parentheses.

25-OHD = 25-Hydroxyvitamin D; PTH = parathyroid hormone; Oc = osteocalcin; NTx = n-terminal telopeptide of type I collagen; Ca/Cr = calcium/creatinine. [a] $p < 0.05$ vs. group A; [b] $p < 0.05$ vs. baseline; [c] $p < 0.05$ vs. controls. The percentage variation (Δ%) in the measured parameters was calculated as: [(measured value – initial value)/initial value] × 100.

Discussion

GH and IGFs with synergic actions control growth, increase muscle mass and affect mineralization of the skeleton [21]. In fact, GH deficiency is associated with decreased bone density in growing children [22].

GH replacement improves BMD in children [22, 23], supporting a physiologic role for GH in increasing the rate of linear growth and bone maturation and in acquisition of bone mass, thus improving final adult height and PBM achievement [21, 24]. PBM, which can be defined as the amount of bone tissue present at the time of skeletal maturation, is an important determinant for osteoporosis and fracture risks in later life.

BMD increases with age and is age-dependent [25] and about half of adult PBM is accumulated during the adolescent growth spurt. The magnitude of the PBM achieved during adolescence depends on genetic-ethnic and environmental factors as physical activity and muscle strength. Also body composition [26] and previous nutrition, especially if calcium-supplemented [9, 10, 25], are important.

It has been demonstrated in the past [9, 25] that calcium supplementation enhanced the rate of increase in total body BMD in prepubertal children. Our data agree with the influence of calcium quantity in the diet for the acquisition of adequate bone mass. In fact, in our calcium-supplemented GHD patients of group B, BMC and BMD were significantly higher after 2 years of treatment than in patients of group A, treated with solely GH. BMC and BMD increase was evident at the legs, but not at the lumbar spine.

The mechanism by which oral calcium increases BMC and BMD remains speculative. However, it is well known that PTH and GH are important regulators, the first of bone remodeling and the second of PTH secretion and target-organ action. Recently, it has been demonstrated that GHD adults, in whom reduced PTH target-organ sensitivity exists, showed after GH replacement therapy PTH decrease, suggesting an improvement in PTH sensitivity [27]. In our study, PTH level tended to increase in the unsupplemented group A and to decrease in the calcium-supplemented group B. In this latter group, calcium elimination, as demonstrated by urine Ca/Cr ratio along the period of GH treatment, showed a trend to decrease, in spite of higher calcium intake. Even if not statistically significant, the behavior of PTH variation and of calcium elimination in group B may be suggestive of a synergistic effect of GH treatment and calcium supplementation on PTH sensitivity and of better calcium repletion. On the other hand, the variations of bone markers during GH therapy reflected those of bone turnover.

OC levels were lower in patients than in controls at the start of treatment; the low levels of this bone formation marker testified in patients a reduction in osteoblastic activity and a failure of accretion of bone mass. OC levels increased after GH therapy as consequence of bone remodeling stimulation, but after 2 years of treatment they were significantly higher in patients supplemented with calcium, testifying a greater apposition of calcium in bones. The behavior of the levels of urinary NTx, marker of bone resorption, was inverse.

Furthermore, since in growing normal children usual calcium intake may not result in maximal mineral retention, we could speculate that in treated GHD children calcium supplementation at the beginning of GH treatment could aid in reaching a major mineral retention, that is particularly evident in those areas of the body that, by means of GH treatment, reach a greater muscle mass and need of a greater bone strength. Therefore, the therapeutic association of GH and calcium could represent a valuable tool in pursuing, besides the final target height, also a proper BMC in GHD patients.

Physical activity is among the factors that promote greater bone density in children and adolescents [28, 29] and it has been positively correlated with the lumbar spine or femoral neck BMD [20, 25]. However, all our patients, calcium supplemented or not, carried out the same physical activity and did not show differences in lumbar BMD.

It is well known that, besides growth and bone mineralization, GH affects body composition [21]. In fact, at the start of treatment, our GHD patients showed increased FM and decreased LTM. During GH substitutive therapy FM decreased and LTM increased and this was confirmed by the constant values of BMI, in spite of the weight's increase [30]. An association of LTM and FM with BMC in children has been demonstrated in the past [26], and more recently it has been stressed the relationship between BMC and LTM using DXA and the importance of LTM in the interpretation of DXA [29, 31, 32]. After 2 years of treatment, in our calcium-supplemented patients of group B, leg BMC and BMD were more positively correlated with regional leg LTM than in the unsupplemented patients of group A. Potential mechanisms for this positive association include the mechanical load and force placed on bone by increased LTM and related skeletal muscles attached to bone [29]. On the other hand, lean body mass reflects skeletal muscle mass and bone and skeletal muscle form an operational unit. As muscle action delivers the largest loads and bone strains, the children with greater muscle mass have a greater BMC [31, 33]. So, bone mass is associated with muscle mass and increase of muscle mass is important for the attainment of PBM [34]. Both GH and calcium, affecting muscle mass and influencing bone cells, could contribute to greater leg bone mass. On the other hand, previous studies showed that the highest contribution of lean mass to BMC was observed at the legs [35], and that calcium supplementation in prepubertal subjects induced a skeletal site selectivity with a greater BMD in the appendicular skeletal sites [10, 36], suggesting an appendicular skeleton more sensitive than the axial skeleton to the effect of calcium supplementation. It will be interesting to discover, continuing in future our study, if calcium supplementation in childhood will be important in maintaining its benefits also in young adults with discontinued GH treatment.

It is frequently stressed that various factors can cause misinterpretation of DXA bone measures in children. Some strategies have been proposed [34] to decrease the influence of growth on the DXA BMD, taking into account various anthropometric parameters to adjust for the influence of body and skeletal growth and development on BMD values. However, these adjustments add great complexity to the pediatric bone studies. On the other hand, it has been recently suggested that DXA BMC is a more reliable measure than DXA BMD for assessing bone acquisition in prepubertal children [34]. Moreover, the two groups of our patients were all prepubertal, even at the final evaluation, and comparable for CA, BA, height, weight and BMI.

In conclusion, in our GHD children treated with recombinant GH, bone densitometric parameters improved, however the group of GHD children supplemented with calcium showed a better BMC and BMD. Calcium supplementation could aid in protecting weight-bearing bones, usually completed in childhood to maximum levels, from risk of osteoporosis and fractures later in life, and in reaching better PBM achievement.

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