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Effects of Treatment with GH Alone or in Combination with LHRH Analog on Bone Mineral Density in Pubertal GH-Deficient Patients

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The aim of the present study was to assess the impact of treatment with GH with or without LHRH analog (LHRH-A) on bone mineralization of GH-deficient adolescents. We studied 17 pubertal, treatment-naive, GH-deficient patients (10 girls and 7 boys) in a prospective, randomized trial. Mean chronological age and mean bone age were 14.1 ± 0.4 and 11.3 ± 0.3 yr, respectively, at the beginning of the study. Treatment with GH + LHRH-A (n = 7) or GH alone (n = 10) started simultaneously. Nutropin was administered at a dose of 0.1 U/kg per day sc until patients reached near final height (NFH), defined as a bone age of 14 yr in girls and 16 yr in boys. Mean time of GH therapy in the patients treated with GH+LHRH-A was 4.8 ± 0.5 yr and in the patients treated with GH alone 2.9 ± 0.7 yr. Lupron was administered at a dose of 300 μg/kg every 28 d im for 3 yr. Bone mineral density (BMD) was assessed yearly by dual-energy x-ray absorptiometry at the lumbar spine (L2-L4) and femoral neck at the beginning of the study, after 3 yr of hormonal therapy, and at NFH. Statistical analysis was performed by *t* test and ANOVA. We observed a significant increase in lumbar and femoral bone mineral content, BMD, SD score, and bone mineral apparent density, compared with baseline in both groups of patients, regardless of whether they were treated with GH alone or in combination with LHRH-A. The patients treated with GH + LHRH-A had a significantly lower bone mineral content after 3 yr of therapy. This difference, however, did not persist after both groups of patients reached NFH. These results indicate that delaying puberty with LHRH-A in GH-deficient patients treated with GH diminishes transient bone mineralization but does not appear to have a permanent impact on BMD. (*J Clin Endocrinol Metab* 87: 84–89, 2002)

OSTEOPOROSIS IS A very common metabolic disorder in North America and around the world. The morbidity and mortality associated with this condition make osteoporosis an important public health problem. Failure to attain a normal peak bone mass during the first two decades of life may be a major risk factor for this disorder. Determinants of peak bone mass are intrinsic factors such as heredity, gender, and hormones and extrinsic factors such as nutrition (intake of calcium, calories, and vitamins) and mechanical influences such as body weight and physical activity. In addition, there are risk factors related to chronic illnesses (such as hypogonadism) and use of drugs such as glucocorticoids or tobacco.

Bone mineral acquisition is maximal during puberty. The influence of hormones such as sex steroids (1–3) and GH has been clearly demonstrated in patients with hypogonadism and GH deficiency (GHD) (4). These patients achieve a decreased bone density, compared with normal individuals, and this deficit is more marked in cases of hypogonadism associated with GHD (5, 6). In contrast, children with precocious puberty have an increase in bone mineral density, compared with age-matched controls, but not when compared with bone age-matched controls (7). LH-releasing hormone (LHRH) analog (LHRH-A) therapy normalizes these parameters in these patients, compared with controls matched by chronological age (7, 8). In cases of hypogonadism or GHD, specific hormone replacement prevents bone loss (6, 9).

GH has clear effects on bone mineralization. GH and IGF-I receptors are present in osteoblasts, and they stimulate cell proliferation and differentiation (10). In addition, GH, probably acting through IGF-I, increases renal 1α hydroxylation of vitamin D and therefore increases calcium and phosphorus absorption in the gut. GH therapy also enhances muscle strength, which influences bone metabolism. In GH-deficient children, diminished bone mineral density as well as decreased biochemical markers of bone formation and resorption have been observed (11–13). However, the degree of osteopenia observed in GH-deficient patients has been variable, probably because of the degree and duration of the GHD in each patient. Nevertheless, GH replacement therapy increases bone mineralization in both children and adults with GH deficiency.

In view of the significant increase in bone mineralization that occurs during puberty, we investigated the effects of GH treatment alone or in combination with LHRH-A on bone mineralization in pubertal GH-deficient patients.

Subjects and Methods

Twenty-one Chilean adolescents with GHD were initially enrolled in this study (9 boys and 12 girls). Their mean initial height was –4.3 ± 1.3 SD score and mean predicted adult height was –3.1 ± 1.2 SD score. Their mean chronological age was 14.3 ± 1.6 yr (range 12–18.5 yr) and mean bone age was 11.3 ± 1.1 yr (range 8.8–13 yr). Mean baseline height velocity was 3 ± 0.3 cm/yr. GHD was defined by clinical criteria, and

Abbreviations: BMAD, Bone mineral apparent density; BMC, bone mineral content; BMD, bone mineral density; GHD, GH deficiency; IGFBP3, IGF-binding protein-3; LHRH-A, LHRH analog; NFH, near final height.

a GH response to two GH stimulation tests (insulin and clonidine) below 7 μg/liter. The GH stimulation tests were performed during early puberty without sex steroid priming. The diagnosis of GHD was made after excluding other identifiable systemic, genetic, skeletal, nutritional, or psychological causes of short stature. The initial clinical characteristics of the 17 patients who completed the study are shown in Table 1.

Ten patients had isolated GHD, six had associated TSH deficiency, and one had combined TSH and cortisol deficiency, which were treated with replacement doses of oral levothyroxine and hydrocortisone. The diagnosis of puberty was based on clinical assessment following the method of Tanner, and a pubertal response to an LHRH test (14). Testicular volume was assessed by the Prader orchidometer. All patients were in early to midpuberty at the beginning of the study (Tanner II to III), with a maximum testicular volume of 10 ml in boys. All girls were premenarcheal before treatment.

The study was approved by the Ethical Review Board of the San Borja-Arriarán Hospital in Santiago, Chile, and at NICHD in Bethesda, Maryland. The study was carried out entirely in Chile. Informed consent was obtained from at least one parent of each patient. All patients were naive to GH and analog therapy before starting their participation in this study. Patients were randomly assigned to receive either GH plus LHRH-A (six girls and four boys) or GH alone (six girls and five boys). Treatment with GH and LHRH-A started simultaneously. GH (Nutropin, donated by Genentech, Inc., South San Francisco, CA) was administered at a dose of 0.1 U/kg per day until achievement of near final height (NFH), defined as a bone age of 14 yr in girls and 16 yr in boys. LHRH-A (depot Lupron, donated by TAP Pharmaceuticals, Inc., Deerfield, IL) was administered at a dose of 300 μg/kg every 28 d for a fixed period of 3 yr (15).

Bone mineral content (BMC) corresponds to the calculated calcium content of a specific bone region that is based on the attenuation of photon energy passing through that bone region. BMC is expressed in grams of hydroxyapatite. Bone mineral density (BMD g/cm^2) of the lumbar spine and femoral neck was measured by dual-energy x-ray absorptiometry, using an Eclipse bone densitometer with a host software revision 2.5.3.a scanner software revision 1.1.4 (Norland Corp., Fort Atkinsons, WI). BMD is calculated by the relation of BMC per area for a specific bone region. Ancillary dual-energy x-ray absorptiometry-derived data were used to calculate bone mineral apparent density (BMAD, g/cm^3) following the model proposed by Katzman et al. (16). BMD results were also expressed as SD score in comparison with sex- and age-matched Chilean controls (17).

Patients were submitted to a complete clinical exam at the beginning of the study and every 3 months, which included height, weight, and body proportions. During each evaluation, height was measured 10 times by the same observer (A.A.) using a Harpenden stadiometer (Holtain Limited, Crymych, UK). Body mass index was calculated as weight/height squared (kg/m^2) and expressed as SD score. Mean daily calcium ingestion was calculated through a patient ingestion self-report. We obtained a bone age at baseline and every 6 months, which was determined by the method of Greulich and Pyle by a single observer blinded to the patient treatment status. In addition, we obtained an early morning (0800 h) serum sample for determination of complete blood count and blood chemistries, sex steroids, thyroid function, cortisol, and gonadotropins (LH and FSH). After obtaining this baseline sample, 100 μg native LHRH iv were administered and serum samples withdrawn at 15, 30, 45, and 60 min for determination of LH and FSH. The interval between the LHRH bolus test and the previous dose of Lupron was approximately 25 d.

Serum LH, FSH, E2, and T were measured by RIA (18, 19). The assay for LH and FSH has a sensitivity of 2 mIU/ml, an interassay coefficient of variation (CV) of 8.5% and 10% respectively, and a intraassay CV of 7% and 8% respectively. For estradiol and testosterone, the detection limits of the assays were 10 pg/ml and 0.1 ng/mL respectively, and the interassay and intraassay CV were 8% and 10%, respectively. Serum GH was measured by a double-antibody RIA (Diagnostic Products Corp., Los Angeles, CA) with a sensitivity of 0.8 ng/ml and an inter- and intraassay CV of 10% and 6.5%, respectively. Serum IGF-I was measured by RIA after acid-ethanol extraction (20), and serum IGF-binding protein-3 (IGFBP3) was measured by immunoradiometric assay using a commercial kit (Diagnostic Systems Laboratories, Inc., Webster, TX). The IGF-I and IGFBP3 detection limits were 10 ng/ml and 0.05 mg/l, respectively, with a interassay CV of 10.2% and 1.8% and an intraassay CV of 8.6 and 1.1%, respectively.

Data are expressed as the mean ± SEM. The statistical analysis of the data was performed by ANOVA.

Results

A report of this study regarding achievement of NFH has been published by our group (21). At the beginning of the study, the mean chronological age, bone age, height, maximum GH concentrations after GH stimulation tests, and

TABLE 1.

	Sex	Chronological age (yr)	Bone age (yr)	Patient's height (SD score)	Mid parental height (SD score)	GH peak ng/ml	IGF-I ng/ml	IGFBP3 mg/liter	Associated disease
GH + LHRH									
1	F	13.0	10.3	−3.5	−1.9	6.4	121	2.5	
2	F	14.0	10.5	−4.8	1.0	2.4	32	1.3	DI, TSH deficient
3	F	13.1	10.7	−3.5	−1.7	0.8	85	1.4	DI, TSH deficient
4	F	14.3	12.5	−2.4	−0.6	5.1	115	1.9	
5	F	15.7	13.0	−5.3	−4.0	0.8	28	1.0	GH deletion
6	M	16.5	12.3	−5.3	−1.6	1.8	16	1.0	Acanthosis, IR
7	M	12.8	11.5	−3.4	Adopted	0.8	40	1.0	Empty sella
Mean		14.2	11.5	−4.0	−1.5	2.6	62	1.4	
SE		0.5	0.4	0.4	0.4	0.9	16	0.2	
GH									
1	F	14.3	8.8	−5.4	−3.2	5.2	46	2.7	
2	F	13.3	12.0	−4.8	−2.4	5.0	111	1.5	
3	F	14.3	10.7	−4.9	−1.0	0.8	33	1.4	Hashimoto thyroiditis
4	F	12.0	10.8	−2.8	−1.8	4.4	115	1.6	
5	F	17.3	11.0	−3.4	−2.0	3.9	35	1.6	TSH deficient
6	M	14.9	11.0	−3.9	−0.7	3.6	141	1.5	DI, TSH deficient
7	M	13.6	11.0	−3.9	−2.7	6.4	126	2.3	
8	M	13.9	11.0	−3.5	−2.2	4.2	129	1.4	TSH deficient
9	M	13.8	11.5	−3.3	Adopted	3.1	124	1.0	Hashimoto thyroiditis
10	M	13.1	12.5	−3.7	−2.0	2.5	75	1.2	TSH + ACTH deficient
Mean		14.0	11.0	−4.0	−2.0	3.9	93	1.6	
SE		0.4	0.3	0.3	0.3	0.5	13	0.1	

DI, Diabetes insipidus; IR, insulin resistance.

body mass index were similar in both groups of patients (Table 1). In addition, mean daily calcium ingestion (580 ± 130 mg) and physical activity were also similar in both groups.

Puberty progression

As expected, mean breast and pubic hair development did not change significantly during the 3 yr of combined GH and LHRH-A therapy, whereas for the group treated with GH alone, puberty progressed at a normal rate. During the study, four patients (two boys and two girls) gradually developed clinical and biochemical evidence of permanent hypogonadism, indicating that the pituitary-gonadal axis was affected, so they were excluded from the study (three in the GH + LHRH-A-treated group and one in the GH-treated group).

As a consequence of gonadotropin and sex steroid suppression, bone age progression was delayed in the group receiving GH + LHRH-A. At the beginning of the study, both groups had a mean height SD score of −4 ± 0.3 SD score, but after finishing GH therapy the group treated with GH + LHRH-A reached a mean near final height of −1.3 ± 0.5 SD score, compared with −2.7 ± 0.3 SD score in the group treated with GH alone ($P < 0.02$) (21).

As expected, menarche was achieved at a significantly older chronological age in the girls treated with GH + LHRH-A (18.2 ± 0.4 yr), compared with the girls treated with GH alone (15.9 ± 0.7 yr). The period elapsed between discontinuation of LHRH-A therapy and menarche was 1.2 ± 0.2 yr, whereas the group treated with GH alone experienced menarche 1.7 ± 0.4 yr after starting GH therapy.

BMD

Lumbar spine. The results of the bone mineral assessment are shown as BMD SD score, compared with gender- and chronological age-matched controls. At baseline, lumbar spine BMD SD score was reduced in the group that received GH alone (−4.4 ± 0.23 SD) as well as in the group that received GH + LHRH-A (−4.1 ± 0.44). The values, however, were appropriate for height age. At NFH, BMD SD score had increased significantly, compared with baseline in the group that received GH alone ($P < 0.001$) as well as in the group that received combined therapy ($P < 0.005$), and the absolute values of BMD SD score were similar in both groups of patients (Fig. 1A). At NFH, BMD SD score, however, were reduced for height age in the group that received combined therapy. After 3 yr and at NFH, the lumbar percent increment in BMD SD score from baseline was similar in both groups of patients.

BMC was similar in both groups of patients at the beginning of the study and did not differ between groups after 3 yr of therapy or at NFH.

BMC within each group increased significantly, compared with baseline after 3 yr of therapy (GH alone, $P < 0.001$, GH + LHRH-A, $P < 0.005$) as well as at NFH (GH alone and GH + LHRH-A, $P < 0.001$) (Fig. 1B).

After the first 3 yr of therapy, the percent increment of BMC, compared with baseline, was significantly different in both groups. The group that received GH alone had a percent increment of BMC, compared with baseline, of 105% ± 11% *vs.* 57% ± 6% in the group that received GH + LHRH-A ($P < 0.005$). This difference in BMC increment, however, did not persist at NFH (104% ± 12% *vs.* 102% ± 10%).

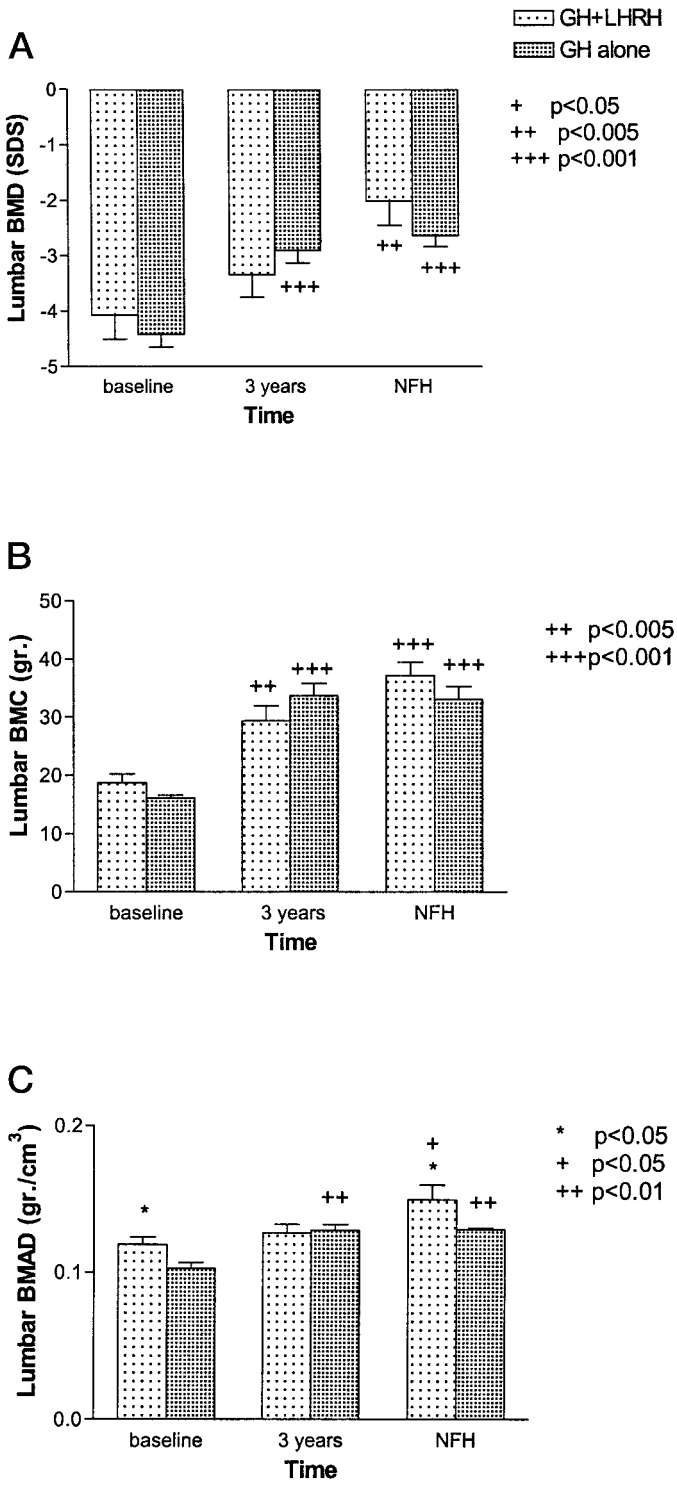

FIG. 1. A, Lumbar BMD SD score at baseline, three years and at NFH. B, Lumbar BMC in grams. C, Lumbar BMAD in grams per cubic centimeter. *, Differences between groups; +, differences within each group, compared with baseline.

Mean values of BMAD at the beginning of the study were significantly higher in the group that received combined

therapy with GH + LHRH-A ($P < 0.05$), but after 3 yr of therapy, there was no difference between both groups of patients. At NFH, however, the group that received GH + LHRH-A had a BMAD of 0.15 ± 0.01 *vs.* 0.13 ± 0.001 g/cm^3 in the group that received GH alone ($P < 0.05$). Lumbar BMAD in the group that received GH alone increased significantly, compared with baseline, after 3 yr ($P < 0.01$) and at NFH ($P < 0.01$). This increase was also significant at NFH for the group that received GH + LHRH-A ($P < 0.05$). These results are shown in Fig. 1C.

The percent increment of BMAD after the first 3 yr of therapy was significantly less in the group that received combined therapy with GH + LHRH-A, compared with the group that received GH alone (6.6 ± 3.3 *vs.* 31.1 ± 4.3, $P < 0.001$). This difference, however, did not persist after reaching NFH.

Femoral neck

At baseline, femoral neck BMD SD score was similarly reduced in the group that received GH + LHRH-A, compared with the group that received GH alone (−2.0 ± 0.5 SD score *vs.* −2.7 ± 0.3 SD score) (Fig. 2A). After the first 3 yr of therapy, the group that received GH alone had a greater, although nonsignificant, femoral neck BMD SD score than the group that received GH + LHRH-A (−0.92 ± 0.35 SD score *vs.* −1.58 ± 0.34 SD), which was still present at NFH. This difference, however, did not reach statistical significance. The increment in BMD SD score after 3 yr of therapy and at NFH was significantly greater in the group that received GH alone, compared with the group that received combined therapy (3 yr 1.76 ± 0.3 *vs.* 0.39 ± 0.3, $P < 0.05$, NFH height 2.24 ± 0.5 *vs.* 0.87 ± 0.4, $P < 0.05$).

Femoral neck BMC was similar in both groups of patients at the beginning of the study and at NFH. After 3 yr of therapy, however, femoral neck BMC was greater in the group that received GH alone, compared with the group that received GH + LHRH-A (3274 ± 276 *vs.* 2340 ± 86 g, $P < 0.05$). In addition, there was a greater gain in BMC in the group that received GH alone after the first 3 yr of therapy (69 ± 17% *vs.* 20 ± 6% $P < 0.05$). This tendency was maintained at NFH but did not reach statistical significance (56.3% ± 10.6% *vs.* 39.5% ± 5.3%), as shown in Fig. 2B. Compared with baseline, BMC after 3 yr of therapy and at NFH improved significantly within each group (Fig. 2B).

Mean values of femoral neck BMAD were similar in both groups of patients at all times during the study, as shown in Fig. 2C. However, after 3 yr of therapy and at NFH, femoral BMAD increased within each group ($P < 0.05$ and $P < 0.005$, respectively). At NFH, femoral BMAD percent increment from baseline was significant in the group that received GH + LHRH-A (19.4 ± 5.4 *vs.* 39.5 ± 5.2, $P < 0.05$).

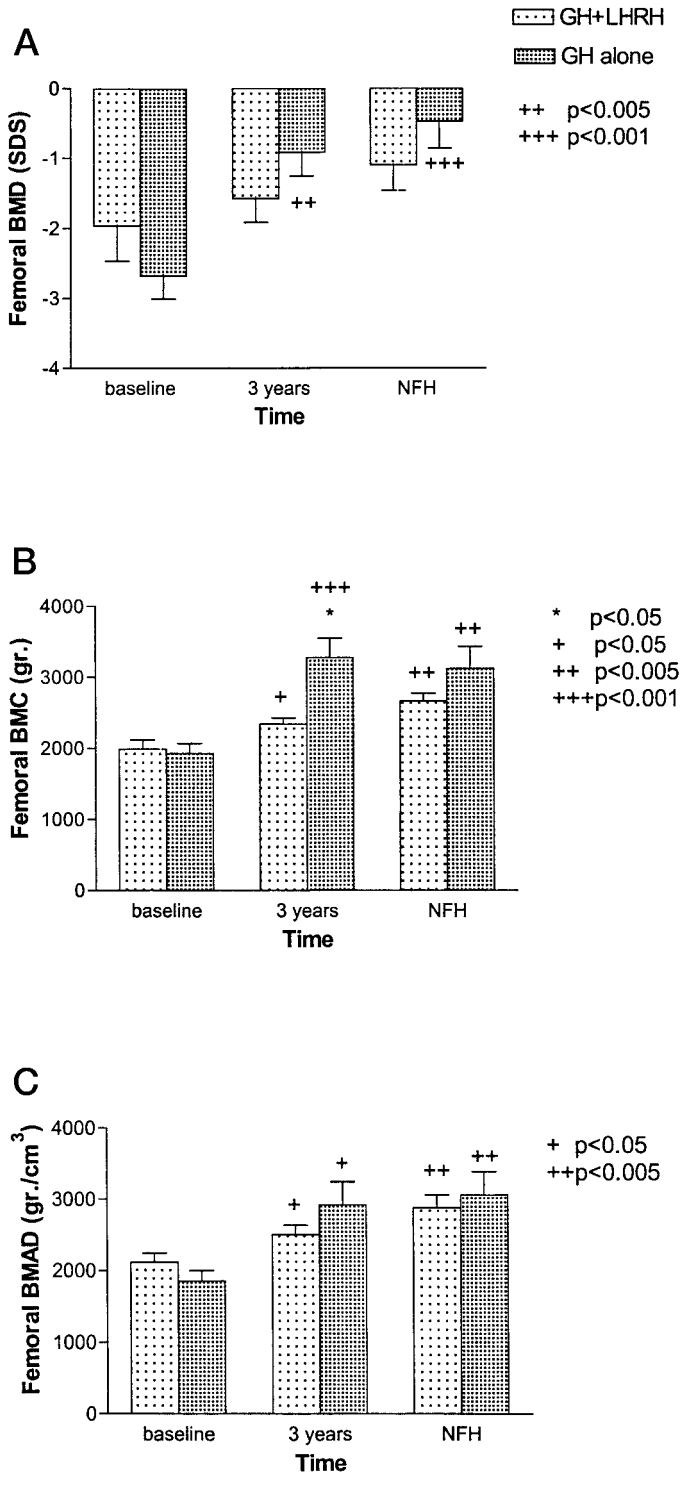

FIG. 2. A, Femoral BMD SD score at baseline, 3 yr and at NFH. B, Femoral BMC in grams. C, Femoral BMAD in grams per cubic centimeter. *, Differences between groups; $^+$, differences within each group, compared with baseline.

Discussion

We report the effects of treatment with GH alone or in combination with LHRH-A on BMD in a randomized, prospective clinical trial in pubertal patients with GHD. The results show that at NFH, there is a significant increase in lumbar and femoral neck BMC, BMD SD score, and BMAD, compared with baseline in both groups of patients. After the initial 3 yr of therapy, however, the percent increment of BMC, BMD SD score, and BMAD in lumbar bone was significantly less in the group that received combined therapy, compared with the group that received GH alone, reflecting the impact of reduced circulating sex steroids on bone me-

tabolism. At NFH, however, there were no differences in the absolute values or percent increment in BMC and BMD SD score, indicating that delaying puberty with LHRH-A in GH-deficient patients treated with GH diminishes transiently bone mineralization but does not appear to have a permanent impact on BMD.

The bone mineralization of the femoral neck, assessed by BMC, BMD SD score, and BMAD increased in both groups of patients during the study, compared with baseline. After the first 3 yr of therapy, however, BMC, the percent increment in BMC, and the increment from baseline for femoral BMD SD score were significantly higher in the group that was treated with GH alone, compared with the group that received combined therapy. These differences did not persist at NFH, except for BMD SD score and the percent increment in BMD SD score, which remained higher in the group that received GH alone. The different impact of therapy on spine and femoral neck bone mineralization might be owing to a differential effect of GH and sex steroids on these bones.

At the time of reaching NFH, all parameters of bone mineralization at the lumbar spine in the group that received GH + LHRH-A were comparable with the group that received GH alone. The group that received treatment with LHRH-A had a longer period of GH exposure because of delayed epiphyseal fusion, compared with the group that received GH alone. Hence, GH by itself may be able to compensate the inadequate bone mineralization induced by the lack of sex steroids.

In the femoral neck, however, the impact of the transient hypogonadal state induced by treatment with LHRH-A did not recover completely, at least when we analyze BMD in terms of BMD SD score. Indeed, BMD SD score in the femoral neck was -1.1 ± 0.36 SD score in the group that received combined therapy with GH + LHRH-A, which had a NFH height of -1.3 SD score, compared with the group that received GH alone, which had a BMD SD score of -0.48 ± 0.38 with a NFH of -2.7 SD score. When we analyze the BMAD, however, which corrects for the height of the patient and the size of the bones, neither the absolute BMAD not the percent increase in BMAD was different between the groups of patients, suggesting that no true difference in BMD SD score was observed.

The strategy of using LHRH-A combined with GH therapy in late-diagnosed pubertal GH-deficient patients increased the length of exposure to GH therapy and augmented the final height of these patients. A recent report (22) has explored another strategy to increase the final adult height of GH-deficient patients, by increasing the GH dose administered. The use of higher GH doses (GH = 0.7 mg/kg per week) increased NFH by 4.6 cm, compared with the group of patients treated with standard doses of GH. An important finding of this study is the normal pace of skeletal maturation observed during high-dose GH therapy, with bone ages advancing approximately 1 yr per each year of chronological age in both the standard and high-dose GH groups. This was also accompanied by a comparable rate of pubertal maturation in both groups of patients. Taken in aggregate, these data indicate that GH therapy, even in relatively high doses, does not unduly advance skeletal maturation or affect the tempo of puberty in GHD children. This conclusion contrasts with that reported by Stanhope *et al.* (23, 24).

In summary, we observed a significant increase in lumbar and femoral BMC, BMD SD score, and BMAD, compared with baseline in both groups of patients, regardless of whether they were treated with GH alone or in combination with LHRH-A. The patients treated with GH + LHRH-A had a significantly lower BMC after 3 yr of therapy. This difference, however, did not persist after both groups of patients reached NFH. These results indicate that delaying puberty with LHRH-A in GH-deficient patients treated with GH diminishes transient bone mineralization but does not appear to have a permanent impact on BMD.

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