

Bone development during GH and GnRH analog treatment

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Abstract

Estrogens, GH and IGFs are essential in the development and growth of the skeleton and for the maintenance of bone mass and density. Treatment of precocious puberty with GnRH analogs (GnRHa), by reducing sex steroid levels, leads to a situation of hypoenestrogenism that may theoretically have a detrimental effect on bone mass during pubertal development. A reduction in bone mineral density (BMD) during GnRHa treatment has been demonstrated, but GnRHa treatment in patients with central precocious puberty (CPP) does not seem to impair the achievement of normal peak bone mass (PBM) at final height. However, calcium supplementation is effective in improving bone densitometric levels and may promote better PBM achievement. In children and adolescents with GH deficiency (GHD), BMD assessed by dual-energy X-ray absorptiometry (DEXA) and bone turnover are significantly reduced, but they are stimulated by GH treatment. GH treatment leads to improved bone density, function of the dose and duration of treatment, and patients may require prolonged GH treatment beyond the time of growth to improve PBM. After the discontinuation of GH therapy, the more active population had higher bone mineral content (BMC) levels than patients with low physical activity. In our experience, the therapeutic association of GH and calcium also represents a valuable tool in pursuing a proper BMC in GHD patients. We concluded that nonhormonal factors, such as physical activity and nutritional factors, are important in determining bone metabolism and bone mass.

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Introduction

Normal bone mineral accretion during childhood and adolescence is a complex process involving genetic determinants, growth hormone (GH)/insulin-like growth factor (IGF)-I effects, gonadal steroids, and nutritional and other environmental factors, such as physical activity (1). In healthy subjects, bone mass increases throughout childhood, with maximal bone mass accrual occurring in early to middle puberty and slowing in late puberty, reaching peak bone mass (PBM), defined as maximal bone mineral density (BMD) (2). About half of the adult PBM is accumulated during the adolescent growth spurt (3–4). The precise age at which PBM is reached is both method- and site-dependent. Most of the lumbar and femoral BMD is achieved around 14.5–16.0 years in girls and 16.5–18.0 years in boys (5–8). Lumbar BMD continues to increase after the completion of growth, achieving PBM 1–2 years later (4, 9). The magnitude of PBM achieved depends not only on genetic potential (race, sex and heredity) (10), but also on nutritional factors (calcium intake) (11, 12), disorders in timing of puberty (3), hormonal deficiency or pharmacologic treatments, and environmental factors as well as physical activity (13, 14).

Estrogens play an important role in skeletal maturation and mineralization and in the relevant increases in bone mass observed during puberty (15). Even at low levels, they promote normal skeletal growth and bone

maturation in boys as well as girls, increasing and maintaining BMD (16), and controlling bone turnover rate (17). Hypoenestrogenic conditions, such as natural menopause and GnRH analogs (GnRHa) administration in premenopausal women (18, 19), are characterized by bone mass reduction. Patients with aromatase deficiency or estrogen-receptor defects have a phenotype that includes tall stature and normal secondary sexual characteristics. These patients have osteoporosis and skeletal immaturity in adulthood despite normal androgen levels (20), with a dramatic improvement in bone density and completion of skeletal maturation after estrogen treatment (21).

GH and IGF are essential in the development and growth of the skeleton and for the maintenance of bone mass and density (22). Levels of GH and IGF-I increase dramatically during normal puberty, augmented by increasing levels of sex steroids. Much of the GH action on bone is mediated through IGF-I, which acts in an endocrine and autocrine/paracrine manner as a bone-trophic hormone that positively affects bone growth and bone turnover by stimulating osteoblasts, collagen synthesis and longitudinal bone growth (23).

In this review we will discuss particularly bone development in two situations: central precocious puberty (CPP) treated by GnRHa and GH deficiency (GHD) in children and adolescents before, during and after GH treatment.

Central precocious puberty (CPP)

The spinal BMD of patients with untreated precocious puberty has been reported to be high for chronologic age (CA) (24) but appropriate for advanced bone age (BA) (25–27), or, in one study, to be lower than the normal mean for BA (28), probably because bone maturation and bone mineralization do not necessarily advance simultaneously (29).

Treatment of precocious puberty with GnRHa, by suppressing gonadotropin secretion and reducing sex steroid levels, leads to hypoestrogenism, which may be accompanied by delayed skeletal maturation and deficient bone mineralization (25, 26). This estrogen deprivation may theoretically have a detrimental effect on bone mass during pubertal development.

CPP during treatment with GnRHa

To test this hypothesis, we studied some years ago (26) bone mineral metabolism and mineralization by dual-energy X-ray absorptiometry (DEXA) before and during treatment in 10 girls aged 6.9–8.4 years affected by CPP and treated with GnRHa, in order to clarify the consequences of estrogen deficiency and of the reduction of GH–IGF-I axis activity.

During treatment, a decrease of serum estradiol levels from pubertal to prepubertal levels was observed. The GH peak following clonidine significantly diminished after 1 year. IGF-I and insulin-like growth factor-binding protein (IGFBP)-3 decreased, though not significantly. Osteocalcin levels, at pubertal range before therapy, decreased to prepubertal levels after 9 and 12 months of treatment, consistently with the arrest of pubertal development, when growth slowed following therapy (30). Urinary hydroxyproline, a marker of bone resorption, significantly decreased after 12 months. Before therapy, lumbar spine and radius bone mass were high for CA, but appropriate for BA; after 12 months of treatment, bone mass in the lumbar spine, but not in the radius, decreased significantly. These changes in bone density may indicate that the initial event, as a consequence of treatment of CPP, is a suppression of bone formation. In our patients, GnRHa administration negatively affected both cortical and trabecular bone, in accordance with another report (25). Cortical bone is less sensitive to rapid changes in bone metabolism, and the rate of bone loss is much lower than that of trabecular bone. The bone loss measured by lateral DEXA, excluding from analysis the posterior portions of the vertebrae (mainly cortical bone), was, in fact, twofold higher than in anteroposterior (AP) scans. When DEXA is used in growing children, an increase of bone size alone induces an increase in photon absorption. When corrected for the volume (vBMD), bone mass significantly decreased. The decline in estrogen levels is associated with increased turnover and bone loss in

adult women, but, in the present study, bone turnover diminished. The bone loss we observed can be explained only by an uncoupling between bone resorption and bone formation. Reduced bone formation is the primary consequence of GnRHa therapy, and this could possibly be related to decreased GH secretion. After the initial reduction of bone formation, there could be, after a few months, a comparable decrease in bone resorption, with consequent transient bone loss.

A reduction in BMD during GnRHa treatment has been demonstrated in other studies (25), although in one study BMD values increased, and the BMD s.d. score for age and skeletal age did not change during treatment (27). It is obvious that, at such a critical age, a decrease in BMD, instead of an increase as expected in normal growing girls, might have a negative impact on PBM and produce a higher risk of postmenopausal osteoporosis. At that time, we stated that this reduction is possibly entirely reversible, as proven in premenopausal women treated with GnRHa (18).

CPP at the end of GnRHa treatment

In a previous study on girls affected by CPP, we demonstrated that BMD reduction during GnRHa therapy was reversible and preventable by providing calcium supplementation from the beginning of treatment (31).

To determine whether GnRHa treatment impaired the achievement of an adequate bone mass at growth completion and whether calcium supplementation improved bone mass in patients treated with GnRHa, we conducted a longitudinal study (32) to evaluate bone mass after long-term GnRHa therapy with or without calcium supplementation in females affected by CPP who had reached final height. In fact, it was demonstrated that calcium intake correlates with bone density in healthy children and adolescents (33), and that calcium supplementation above the recommended dietary allowances increases bone density in children (34).

We studied 48 Caucasian females affected by CPP (age at diagnosis, 7.19 ± 0.96 years), randomly assigned to two groups: group A ($n = 21$) treated with GnRHa and group B ($n = 27$) treated with GnRHa plus calcium gluconolactate and carbonate (1 g calcium/day in two doses) for at least 2 years. Auxologic parameters (standing height, weight, body mass index) and BMD at the lumbar spine (L2–L4, anteroposterior (AP)–BMD; lateral BMD; and volumetric (vBMD)), total BMC (TBMC) and total BMD (TBMD), by DEXA were evaluated at the beginning, at the end of treatment and at final height.

The vBMD was significantly higher in group B than in group A at the end of the treatment period and at final evaluation ($P < 0.05$). The percent change ($\Delta\%$) between the start and end of treatment period in AP–BMD and vBMD was significantly higher in group B

than in group A and also between the start of treatment and final evaluation (Table 1).

In all our females with CPP (both groups A and B) at final evaluation, treated with GnRH_a, bone densitometric parameters (final TBMC, TBMD, AP-BMD L2-L4 and vBMD) were in the normal range for age and sex, even if lower than in controls, but the differences were not statistically significant (Fig. 1). Therefore, in our study, as in other studies (35–38), GnRH_a treatment in patients with CPP does not seem to impair the achievement of normal PBM. In calcium-supplemented patients, vBMD levels at the end of therapy and final evaluation were significantly higher than in patients treated only with GnRH_a. Moreover, $\Delta\%$ AP-BMD and $\Delta\%$ vBMD between the beginning of treatment and final evaluation were also significantly higher in calcium-supplemented patients than in patients treated only with GnRH_a, with a significant relationship with the duration of calcium supplementation. Thus, calcium supplementation is effective in improving bone densitometric levels and may preserve better PBM achievement (32).

A limitation of the study is that, as well known, PBM is not achieved at final height but later in life (2). Therefore, the effect of precocious puberty and its treatment on PBM should be re-evaluated later, at approximately 20–30 years of age.

Probably the majority of the general population do not require calcium supplementation because of good genetic background, adequate diet, and good physical activity. But in some individuals, such as patients treated with drugs that potentially interfere with bone mineral metabolism, it is probably better to increase calcium intake (2).

GnRH_a treatment outside CPP

In a randomized clinical trial, treatment with GnRH agonist was used to increase adult height in adolescents with short stature and normally timed puberty (39). In this

study, the principal adverse event in the GnRH-agonist group was reduced lumbosacral BMD during treatment and inadequate catch-up accretion of bone mineral after treatment (mean lumbar vertebral BMD at the time adult height was achieved, 1.6 ± 1.2 s.d. below population mean, vs 0.3 ± 1.2 s.d. below population mean in the placebo group; $P < 0.001$). Due to this adverse effect, the authors stated that such treatment cannot be routinely recommended to augment height in adolescents with normally timed puberty.

GH deficiency (GHD)

In children and adolescents with GHD, BMD assessed by DEXA and bone turnover are significantly reduced (40), reflecting decreased bone modeling and remodeling (41), because of delayed bone maturation or absence of GH anabolic activity (42).

Reduced BMD detected by DEXA is commonly reported in untreated adult patients with childhood (43, 44) or adult onset GHD (45–46) (the former showing more pronounced effects of GHD on bone mass than the latter), with respect to normal controls and treated patients, and one-third of patients had lumbar bone density 2 s.d. or more below the normal mean (48).

Reduced BMD by DEXA in GHD adults may be explained either by a failure to achieve normal PBM or by subsequent bone loss during adult life (45, 46), and the severity of bone loss is proportional to the biochemical severity of GHD (49).

However, DEXA provides an areal density measurement rather than a true volumetric density, and low bone density measurements may reflect reduced height and thus bone size in these patients. When bone density is measured by peripheral quantitative computed tomography (pQCT) or estimated by calculated methods from DEXA measurements to correct for bone size (vBMD), spinal bone density is still reported as reduced if compared with age- and sex-matched reference data (50, 51). However, in recent studies,

Table 1 Chronologic age (CA) and bone age (BA) of patients at various times of evaluation: densitometric values (volumetric bone mineral density–vBMD), $\Delta\%$ AP-BMD and $\Delta\%$ vBMD between the start and end of treatment (start–stop), end of treatment and final evaluation (stop–final), and the start of treatment and final evaluation (start–final).

		Start of treatment	End of treatment	Final evaluation
CA		7.3±0.9	11.3±0.97	16.2±1.9
BA		8.80±1.24	12.35±0.43	16.93±0.98*
vBMD g/cm ³	group A	0.175±0.016	0.192±0.021	0.227±0.024
	group B	0.177±0.014	0.213±0.022 ^a	0.246±0.023 ^b
		Start–end of treatment	End of treatment–final evaluation	Start of treatment–final evaluation
$\Delta\%$ AP-BMD	group A	16.16±1.90	40.81±2.45	56.97±1.45
	group B	20.36±1.10 ^a	40.87±3.32	61.23±1.61 ^a
$\Delta\%$ vBMD	group A	9.26±5.15	17.18±3.05	28.01±5.76
	group B	19.08±3.52 ^a	14.81±3.08	36.69±5.01 ^a

* In each case > 15 years.

^a $P < 0.01$ vs group A.

^b $P < 0.05$ vs group A.

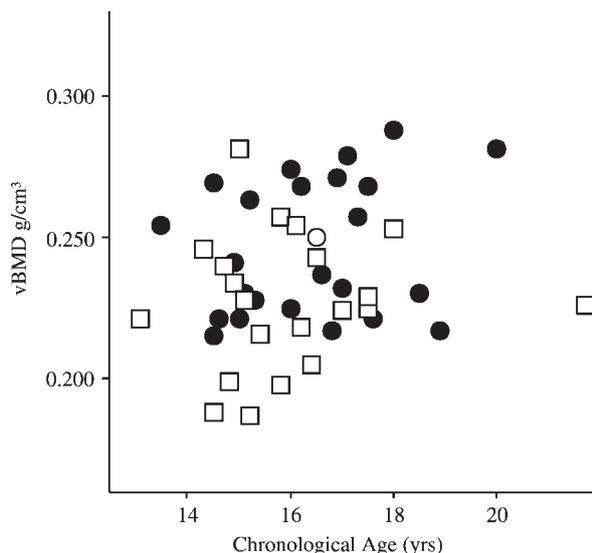


Figure 1 Central precocious puberty (CPP). Individual vBMD levels at final evaluation in group A (GnRH alone, white squares) and group B (GnRH and Ca, black circles). vBMD levels were significantly higher in group B than in group A ($P < 0.05$).

pQCT showed that cortical bone density was not reduced in GHD children (52), and vBMD was reported as normal to near normal (mean Z scores: -1.2 , $+0.8$, and $+0.8$ for lumbar spine, femoral neck and total skeleton respectively) in patients with genetic growth hormone-releasing hormone (GHRH)-receptor deficiency (53). These reports stressed that GH/IGF-I deficiency has relatively little impact on bone mineralization during the bone accretion phase, in marked contrast to its effect on bone elongation and overall bone size.

Adult-onset GHD patients have a threefold increase in fracture risk compared with the general population (54), and it was recently demonstrated that children with GHD had an approximately fourfold decreased fracture frequency from diagnosis up to final height compared with controls (61).

GH treatment

GH treatment in GHD children stimulates bone turnover (40–42) and improves BMD (42, 55–58).

In treated GHD children, an increase in lumbar bone density by DEXA after 6 months of treatment, but no significant change in calculated vBMD until after 2 years of treatment, was reported. Early changes in bone density measurements may reflect changes in bone size, but prolonged treatment results in improvement in net bone formation (42). Longitudinal data in GHD children treated for an average of 4 years demonstrated a significant improvement in radial and lumbar bone density. The greatest improvement was observed with the longest treatment duration, with z-scores approaching mean reference values (58).

At final height, normal (60, 61) or reduced (62) mean values of lumbar BMD were found in GH-treated GHD patients. Approximately 20% of GHD children treated with GH had a value of lumbar BMD between -1 and -2 s.d. of the normal mean at final height, with increased susceptibility to fractures during GH treatment (61). Reduced lumbar BMD was found especially in patients with GHD who had received interrupted and low-dose GH treatment during the period of pituitary-derived GH during childhood (44, 59), but it has been shown that there was no difference in lumbar BMD at final height between patients with GHD who had received standard (0.3 mg/kg per week) or high GH dosage (0.7 mg/kg per week) (62).

In patients with GHD, increased lumbar BMD after the attainment of final height has been found 2 years (60) after discontinuation of GH treatment. GH-retreated patients with childhood (63, 64) or adult (65) onset GHD also had an increased BMD after discontinuation of GH treatment that could be due to a persisting effect of GH treatment inducing a bone-remodeling cycle, which continues until the new bone is fully mineralized (65).

In adolescents with GHD who normally discontinue GH at completion of linear growth, BMD is substantially lower than PBM for a young adult population. A recent report on adolescents with GHD who continued GH administration after the completion of linear growth showed an increased lumbar BMD after 12 months of therapy, which was not observed in untreated patients (66). Moreover, reinstatement of GH replacement after final height in severely GH-deficient patients induced significant progression toward PBM (67, 68).

All these data suggest that GH treatment leads to improved bone density, according to the dose and duration of treatment, and that patients may require prolonged GH treatment beyond the time of growth to improve PBM. Cessation of GH at achievement of final height, by limiting PBM, may predispose to clinically significant osteoporosis in later life, also by a superimposed accelerated loss of BMD with advancing age similar to the situation observed in adult-onset GHD.

Pubertal GH-deficient patients treated with GH and GnRH had a significantly lower BMC after 3 years of therapy. This difference, however, did not persist after both groups of patients reached final height.

GH treatment and calcium supplementation

We conducted a preliminary study to evaluate BMD during GH therapy, with or without calcium supplementation, to determine whether calcium supplementation improved bone mass in patients treated with GH and calcium.

Twelve prepubertal Caucasian GH-deficient patients (seven females, five males), 5–14 years old, took part in the study. The diagnosis of GHD was based on the

following criteria: height of ≤ 2 SDS; bone age delay of > 2 years compared with CA; peak GH of < 10 ng/ml in at least two consecutive pharmacologic tests; reduced IGF-I. None of the patients had organic GHD or multiple pituitary hormone deficiency, all being affected by idiopathic isolated GHD, as assessed by full endocrine evaluation and pituitary molecular resonance imagery (MRI).

Patients, all treated with GH at a dose of 0.033 mg/kg per day 6 days a week, were randomly assigned to two groups (A and B) comparable for age, BA weight and height, using a computer pseudo-random number generator. Patients in group A ($n = 6$) were treated solely with GH; patients in group B ($n = 6$) received GH plus supplementation of calcium gluconolactate and carbonate (1 g calcium/day *per os* in two doses).

No patients received other drugs known to interfere with bone mineral metabolism. All the subjects were instructed to continue their usual physical activity and diet, thereby ensuring adequate caloric (70–80 cal/kg per day), protein (> 1 g/kg per day), calcium (> 800 mg/day), and phosphate (> 800 mg/day) intake during treatment. Diet and dietary calcium intake were investigated by a weighed food record and exercise by an exercise diary. Compliance with assumption of calcium supplementation was checked by a diary.

At the start of therapy, the patients of groups A and B were comparable for age, weight, height and body composition, BMC and bone turnover. The biochemical data of bone turnover of patients of groups A and B after 12 months of treatment evidenced comparable values in both groups. After 12 months of therapy, BMC significantly increased in patients of group B, supplemented with calcium, compared with those of group A treated solely with GH ($P < 0.05$).

Therefore, in our experience, the therapeutic association of GH and calcium represents a valuable tool in pursuing not only the final target but also proper BMC in GHD patients.

Physical activity at the end of GH treatment

For better determination of body composition after discontinuation of GH therapy, we re-evaluated 20 young men with GHD diagnosed in childhood that had completed pubertal development (age 18–20 years), 12 months after stopping therapy for at least 6 years with GH at the dose of 0.6 IU/kg per week. The parameters studied included final height, spontaneous nocturnal GH secretion and body composition by DEXA (Expert XL; Lunar Corp., Madison, WI, USA), which enabled assessment of whole-body as well as regional soft tissue composition. Re-evaluation of GH secretion in these patients showed that 12 remained GH deficient (persistent GHD) with abnormal spontaneous nocturnal GR secretion (11 multiple pituitary deficiency and one isolated GHD), while eight recovered

normal somatotrophic secretion (transient GHD). BMC was positively influenced, as expected, by GR action. GHD patients, 12 months after discontinuation of GH therapy, had BMC levels very close to the control group of normal young adults. One year after stopping the GH treatment, patients with confirmed GHD showed an increased fat mass as compared with the value at the end of the treatment; in this group, we divided patients into two distinct populations, on the basis of exercise quantity. The more active population had higher BMD levels, especially at lumbar and thoracic spine than the patients with low physical activity (Table 2) (69). We concluded that nonhormonal factors, such as physical activity and nutritional factors, are important in determining bone metabolism and bone mass.

Conclusions

Normal bone mineral accretion during childhood and adolescence is a complex process involving genetic determinants, GH/IGF-I effects, gonadal steroids, and nutritional and other environmental factors, such as physical activity. Estrogens, GR and IGFs are essential in the development and growth of the skeleton and for the maintenance of bone mass and density.

Treatment of precocious puberty with GnRHa, by suppressing gonadotropin secretion and reducing sex steroid levels, leads to a situation of hypoestrogenism, which may theoretically have a detrimental effect on bone mass during pubertal development. A reduction in BMD during GnRHa treatment has been demonstrated, but GnRHa treatment in patients with CPP does not seem to impair the achievement of a normal PBM at final height. However, calcium supplementation is effective in improving bone densitometric levels and may promote better PBM achievement.

In children and adolescents with GH deficiency, BMD by DEXA and bone turnover are significantly reduced, but are stimulated by GH treatment. At final height, normal or reduced mean values of lumbar BMD were found in GH-treated GHD patients. GH treatment leads to improved bone density, function of the dose

Table 2 Bone mineral density (g/cm²) in GHD patients 12 months after discontinuation of GH therapy, subdivided by physical activity (high and low).

	High	Low
Number	7	5
Subtotal	1.082 \pm 0.043	0.870 \pm 0.037
Lumbar spine	1.187 \pm 0.073	0.742 \pm 0.069
Thoracic spine	1.004 \pm 0.039	0.649 \pm 0.042
Left leg	1.288 \pm 0.086	1.056 \pm 0.065
Right leg	1.273 \pm 0.084	1.043 \pm 0.038
Left arm	0.787 \pm 0.024	0.673 \pm 0.033
Right arm	0.802 \pm 0.022	0.675 \pm 0.036

and duration of treatment, and patients may require prolonged GH treatment beyond the time of growth to improve PBM. After the discontinuation of GH therapy, the more active population had higher BMC levels than patients with low physical activity. In our experience, the therapeutic association of GH and calcium also represents a valuable tool in promoting proper BMC in GHD patients.

We concluded that nonhormonal factors, such as physical activity and nutritional factors, are important in determining bone metabolism and bone mass.

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