Bone Mass at Final Height in Precocious Puberty after Gonadotropin-Releasing Hormone Agonist with and without Calcium Supplementation

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The aim of our longitudinal study was to evaluate bone mass in girls affected by central precocious puberty (CPP) that have reached final height, treated with GnRH agonist triptorelin (GnRHa), with or without calcium supplementation. We studied 48 Caucasian females affected by CPP (age at diagnosis, 7.19 ± 0.96 yr), randomly assigned to two groups: group A (n = 21) treated with GnRHAs and group B (n = 27) treated with GnRHAs plus calcium gluconate and carbonate (1 g calcium/day in two doses) for at least 2 yr. Auxological parameters (standing height, weight, body mass index) and bone mineral density (BMD) at the lumbar spine (L2-L4, anteroposterior (AP)-BMD; lateral BMD; volumetric (v)BMD) by dual-energy x-ray absorptiometry were evaluated at the beginning (chronological age (CA), 7.29 ± 0.91 yr; bone age (BA), 8.80 ± 1.24 yr) and at final height (CA, 11.27 ± 0.97 yr; BA, 12.35 ± 0.43 yr) and at final height (CA, 16.17 ± 1.9 yr; BA, 16.93 ± 0.88 yr, in each case >15 yr). Total bone mineral content, total BMD, and fat percentage were evaluated at the end of the study period using dual-energy x-ray absorptiometry. Final height was significantly higher than predicted height at diagnosis (159.9 ± 6.3 cm vs. 152.9 ± 9.6 cm; P < 0.05). Body mass index and fat percentage were not statistically different from control values. Densitometric values at final evaluation in groups A and B together were lower than in controls, but the differences were not statistically significant. The vBMD was significantly higher in group B than in group A at the end of treatment period (0.213 ± 0.022 g/cm² vs. 0.192 ± 0.021 g/cm²; P < 0.01) and at final evaluation (0.246 ± 0.023 g/cm² vs. 0.227 ± 0.024 g/cm²; P < 0.05). The percentage change (Δ%) between the start and end of treatment period in AP-BMD and vBMD was significantly higher in group B than in group A (Δ% AP-BMD: 20.36% ± 1.10% vs. 16.16% ± 1.90%, P < 0.01; Δ% vBMD: 19.08% ± 3.52% vs. 9.26% ± 5.15%; P < 0.01) and also between the start of treatment and final evaluation (Δ% AP-BMD: 61.23% ± 1.61% vs. 56.97% ± 1.45%, P < 0.01; Δ% vBMD: 36.69% ± 3.01% vs. 28.01% ± 3.76%, P < 0.01). In all our females with CPP treated with GnRHa, bone densitometric parameters were in the normal range for age and sex. However, bone mass achievement seemed to be better preserved in the group of patients supplemented with calcium. (J Clin Endocrinol Metab 88: 1096–1101, 2003)

It is well known that bone mineral density (BMD) increases with age and puberty is a crucial period for bone development and peak bone mass (PBM) achievement (1). The genetic potential for bone accumulation could be limited not only by insufficient calcium intake and inadequate physical activity but also by disruption of the pubertal calendar (2). Treatment of precocious puberty with GnRH agonists (GnRHa) (3), by suppressing gonadotropin secretion and reducing sex steroid levels, may have a detrimental effect on bone mass during pubertal development (4, 5). On the other hand, it has been demonstrated that calcium intake correlates with bone density in healthy children and adolescents (6) and calcium supplementation above the recommended dietary allowances increases bone density in children (7, 8).

The aim of the present longitudinal study was to evaluate bone mass after long-term GnRHa therapy with or without calcium supplementation in females affected by central precocious puberty (CPP) who have reached final height 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.

Patients and Methods

Patients

We investigated 48 Caucasian girls affected by CPP. Informed consent was obtained from the parents of each girl before starting the study protocol, and local hospital ethical committee approved the study.

Diagnosis of CPP was based on the appearance of pubertal signs (breast and pubic hair at stage II or above, according to Tanner) before 8 yr of chronological age (CA) (appearance of pubertal signs: CA, 6.94 ± 1.05 yr; age range, 4.5–7.9 yr); bone age (BA) more than 1 yr beyond CA; uterus longitudinal diameter (detected by ultrasonography) greater than 3.5 cm; LH and FSH responses to the GnRH stimulation test (100 mg/m², iv bolus dose); and estradiol concentrations in the pubertal range (9).

None of the patients had evidence of progressive organic disorders in the central nervous system detected by computed tomography or magnetic resonance imaging, identifiable adrenal or gonadal pathology, or thyroid deficiency or had previously been treated with inhibitory steroids. Renal and hepatic functions were normal.

After CPP diagnosis, patients were assigned to treatment with the long-acting GnRHa triptorelin (Decapeptyl, IPSEN, Milan, Italy) at a dose of 3.75 mg, im, every 28 d (0.123 ± 0.12 mg/kg; range 0.10–0.15 mg/kg), or placebo (control group).

Abbreviations: AP-BMD, Anteroposterior bone mineral density; BA, bone age; BMD, bone mineral density; BMI, body mass index; CA, chronological age; CPP, central precocious puberty; Δ%, percentage variation; DXA, dual-energy x-ray absorptiometry; GnRHa, GnRH agonist; L-BMD, lateral BMD; PAH, predicted adult height; PBM, peak bone mass; % FAT, fat percentage; TBMC, total body bone mineral content; TBMD, total BMD; TH, target height; vBMD, volumetric BMD.
mg/kg) for a period of 3.97 ± 1.14 yr (range, 2.1–4.6 yr). CA at diagnosis was 7.19 ± 0.96 yr; CA and BA at the start of therapy were 7.29 ± 0.91 yr and 8.80 ± 1.24 yr, respectively.

Patients were randomly assigned to two groups (A and B) comparable for age, BA, height, and weight using a computer pseudorandom number generator. Patients in group A were treated with GnRHa (n = 21) for a period of 4.08 ± 1.28 yr; patients in group B (n = 27) received a treatment with GnRHa for 3.88 ± 1.31 yr plus supplementation of calcium gluconolactate and carbonate (1 g calcium/day in two doses) during GnRHa treatment for a period of 2.89 ± 0.59 yr and in each case >5 yr (range, 2.1–4.6 yr).

In group A patients, the onset of pubertal signs was at CA of 6.88 ± 1.21 yr and the CA at diagnosis was 7.18 ± 1.13 yr. In group B patients, the onset of pubertal signs was at 6.98 ± 1.12 yr and CA at diagnosis was 7.20 ± 1.09 yr.

No patient 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 kcal/kg-d), protein (>1 g/kg-d), calcium (>800 mg/d), and phosphate (>800 mg/d) intake during treatment. Diet and dietary calcium intake in particular were investigated by a weighed food record and exercise by an exercise diary. Compliance in assumption of calcium supplementation was checked by the same expert observer throughout the study.

The 48 subjects were evaluated at the end of therapy, at CA of 11.27 ± 0.97 yr (range 9.81–12.73) and BA of 12.35 ± 0.43 yr (range 11.5–14 yr) and with a final evaluation when they reached final height, at CA of 16.17 ± 1.9 yr (range 13.1–21.7 yr) and BA of 16.93 ± 0.98 (in each case >15 yr).

Calcium supplementation was not continued after the stop of GnRHa treatment. Patients and families of both groups were educated to have an adequate calcium intake. Mean interval time between stop of treatment and final evaluation was 4.9 ± 1.4 yr for total group of patients (range 1.6–7.7 yr), 4.7 ± 1.8 yr for group A (range 1.6–7.7 yr), and 5.1 ± 1.6 for group B (range 2.3–6.9 yr). All calcium-supplemented patients were evaluated at least 2 yr after supplementation was stopped.

During the final evaluation, age at menarche and menstrual pattern were investigated.

Methods

Standing height, weight, body mass index (BMI), BA, and BMD at the lumbar spine (L2-L4) were evaluated at the start and end of GnRHa treatment and at final evaluation. Total body bone mineral content (TBMC), total BMD (TBMD) and fat percentage (% FAT) were evaluated at the final evaluation.

Standing height was measured using a Harpenden stadiometer (Holtain Ltd., Crymlyn, UK). BMI was calculated as weight (kilograms)/height (square meters) and compared with age- and sex-matched reference values (10) to calculate SD score (SDs). BA evaluation was determined blindly by the same expert observer according to the Greulich and Pyle method (11) and expressed in years. Predicted adult height (PAH) at diagnosis, based on height and BA, was calculated by the Bayley and Pinneau method (12). Target height (TH) was calculated from the mean height of the parents adjusted for sex, as described by Tanner et al. (13). Height was considered as final adult stature when BA was equal to or greater than 15 yr and the patient’s growth rate was less than 0.5 cm/yr during the preceding year.

TBMC (grams), TBMD (grams per square centimeter), and BMD at the lumbar spine were measured using dual-energy x-ray absorptiometry (DXA). BMD at the lumbar spine was assessed at diagnosis and the end of treatment by Sophos DXA (Sophos L-XRA 3.1, Sopha Medical S.N.L, Les Ulis, France). BMD at the lumbar spine, TBMC, and TBMD at the final evaluation were assessed using DXA (Expert XL; Lunar Corp., Madison, WI).

The second, third, and fourth lumbar vertebrae were scanned by anteroposterior projection (AP-BMD) and lateral scan (L-BMD). DXA-derived data were used to calculate lumbar spine volumetric BMD (vBMD), expressed in grams per cubic centimeter, taking the vertebral body as an ellipsoid cylinder and dividing bone mineral content obtained by lateral scan (in grams) by body vertebral volume (in cubic centimeters), calculated (π × width/2 × depth/2 × height) to reduce the confounding effect of bone size (14). Vertebral dimensions (anterior width, depth, and height) were obtained using software data. A cross-calibration between the two DXA lumbar spine instruments was obtained for anteroposterior and lateral scan using morphologic commercial phantom (Hologic, Inc., Waltham, MA). To compare densitometric data, we applied the conversion factors Sophos-Lunar (Bertoldo, F., unpublished data)—AP-BMD: Lunar (estimated) = 0.98021 (Sophos) 0.004112; L-BMD: Lunar (estimated) = 0.97274 (Sophos) 0.003988; vBMD: Lunar (estimated) = 0.97165 (Sophos) 0.003899.

DXA was calibrated daily using a commercial phantom to exclude measurement drifts during the study period. Coefficients of variation were: 1.1% AP-BMD, 1.8% L-BMD, 2.6% vBMD for Sophos instrument and less than 1% AP-BMD, 1.2% L-BMD, and 2.1% vBMD for Lunar Corp. instrument, according to the manufacturers. Serial measurements of phantom were routinely performed during the study. Precision error for both Sophos and Lunar Corp. instruments was less than 1% during the study. TBMC, TBMD, and % FAT were measured with Expert XL (Lunar Corp.). Coefficients of variation were 0.6% for TBMC, 1.0% for TBMD, 2.2% for fat, according to the manufacturer.

AUXOLOGICAL, body composition, and bone densitometric data were compared with control groups of the same CA (Tables 1 and 2), with BA appropriate for CA, BMI between the 25th and 75th percentile (10), normal intake of calcium and phosphate, and normal physical activity. The percentage variation (Δ%) in the measured parameters was calculated as: [(measured value − initial value)/initial value] × 100.

Statistical analysis

Results are expressed as means ± sd. Statistical analyses were performed using unpaired t test, ANOVA, and simple regression analysis. All statistical analyses were performed using a data analysis system (StatView 4.5; Abacus Concepts, Inc., Berkeley, CA) run on an Apple PowerMac computer (Apple Computer, Inc., Cupertino, CA). Statistical significance was set at P < 0.05.

Results

Clinical data regarding patients at the start, end of treatment, and final evaluation are reported in Table 1. In all patients, TH and PAH at the start of treatment were 161.9 ± 6.3 and 152.9 ± 9.6 cm, respectively. Final height was 159.9 ± 6.2 cm (P < 0.05 vs. PAH); it was within TH in 81% of all patients. Differences between the two groups were not found (Table 1).

After the stop of treatment, a prompt recovery of hypothalamic-pituitary-gonadal axis activity was seen and the response to the GnRH stimulation test returned pubertal after 6–9 months. Menarche or menarche started at age 12.4 ± 0.9 yr (range 10.9–14.2 yr) in all patients with no statistical difference between the two groups; no patient showed pathologic menstrual pattern.

BMI and BMD expressed as SD scores at pretreatment, the end of treatment, and final evaluation were not significantly different, with no statistically significant differences among the two groups and controls (Table 1). % FAT, determined by DXA, was 30.99% ± 3.95% for the total group of patients, 30.75% ± 4.06% in group A, and 31.18% ± 3.88% in group B, with no statistical significance among the two groups and controls (30.51% ± 5.75%).

There were no differences in exercise levels or exposure to sunlight between the two groups of subjects, as reported in each patient’s food and exercise diary. The compliance in calcium supplementation assumption in group B was more than 84%. Calcium intake was 897 ± 95 mg/d in group A, 1510 ± 168 mg/d in group B (during the period of calcium supplementation), and 907 ± 108 mg/d in the control group.
TABLE 1. Clinical data of all girls affected by CPP (total) and controls, of those treated with GnRHa alone (group A), and of those treated with both GnRHa and calcium (group B), at the start and at the end of therapy and at the final evaluation

<table>
<thead>
<tr>
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<th>Start of treatment</th>
<th>End of treatment</th>
<th>Final evaluation</th>
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<tbody>
<tr>
<td></td>
<td>Total (n = 48)</td>
<td>Group A (n = 21)</td>
<td>Group B (n = 27)</td>
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<tr>
<td></td>
<td></td>
<td>Group A (n = 21)</td>
<td>Group B (n = 27)</td>
</tr>
<tr>
<td>CA (yr)</td>
<td>7.29 ± 0.91</td>
<td>7.14 ± 1.44</td>
<td>7.31 ± 0.98</td>
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<td>BA (yr)</td>
<td>8.80 ± 1.24</td>
<td>8.82 ± 1.04</td>
<td>8.77 ± 1.02</td>
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<tr>
<td>Height (cm)</td>
<td>130.8 ± 8.7</td>
<td>129.9 ± 6.8</td>
<td>131.1 ± 7.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>30.8 ± 6.4</td>
<td>30.4 ± 5.1</td>
<td>30.9 ± 6.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.9 ± 2.5</td>
<td>17.8 ± 2.4</td>
<td>17.9 ± 2.3</td>
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<tr>
<td>BM (SD values)</td>
<td>0.40 ± 0.21</td>
<td>0.39 ± 0.26</td>
<td>0.41 ± 0.31</td>
</tr>
<tr>
<td>PAH (cm)</td>
<td>152.9 ± 9.6</td>
<td>153.3 ± 4.8</td>
<td>152.3 ± 6.9</td>
</tr>
<tr>
<td>Target height (cm)</td>
<td>160.8 ± 6.3</td>
<td>160.8 ± 6.3</td>
<td>162.5 ± 6.5</td>
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Data from controls are at the same CA of patients at the same points. PAH, predicted adult height at diagnosis, based on height and bone age (Bayley and Pinneau method).

TABLE 2. DXA data in total group, group A (GnRHa), and group B (GnRHa + Ca), at the start of therapy, at the end of therapy, and at the final evaluation

<table>
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<tr>
<th></th>
<th>Start of treatment</th>
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<tr>
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<tr>
<td></td>
<td></td>
<td>Group A (n = 21)</td>
<td>Group B (n = 27)</td>
</tr>
<tr>
<td>TBMD (g)</td>
<td>1835.9 ± 181.5</td>
<td>1802.1 ± 132.5</td>
<td>1862.2 ± 210.8</td>
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<tr>
<td>TBMD (g/cm²)</td>
<td>1.103 ± 0.049</td>
<td>1.093 ± 0.039</td>
<td>1.111 ± 0.055</td>
</tr>
<tr>
<td>AP-BMD (g/cm²)</td>
<td>0.630 ± 0.058</td>
<td>0.631 ± 0.059</td>
<td>0.629 ± 0.070</td>
</tr>
<tr>
<td>L-BMD (g/cm²)</td>
<td>0.547 ± 0.047</td>
<td>0.545 ± 0.048</td>
<td>0.548 ± 0.051</td>
</tr>
<tr>
<td>vBMD (g/cm³)</td>
<td>0.176 ± 0.015</td>
<td>0.175 ± 0.016</td>
<td>0.177 ± 0.014</td>
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</table>

Data from controls are at the same CA of patients at the same points.

a vBMD was significantly higher (P < 0.01) at the end of therapy in group B than in group A.

b vBMD was significantly higher (P < 0.05) at final evaluation in group B than in group A.
Table 2 shows DXA evaluation in total group, group A, group B, and controls

Bone densitometric values (TBMC, TBMD, AP-BMD, LB-MD, and vBMD) in all patients (groups A and B together) were lower than in controls, but the differences were not statistically significant for all parameters studied.

The vBMD levels were significantly higher in group B than in group A at the end of therapy (0.213 ± 0.022 g/cm² vs. 0.192 ± 0.021 g/cm², *P* < 0.01) and final evaluation (0.246 ± 0.023 g/cm² vs. 0.227 ± 0.024 g/cm², *P* < 0.05) (Fig. 1).

The ∆% in AP-BMD and vBMD were significantly higher in group B than in group A between the beginning and the end of treatment (∆% AP-BMD: 20.36% ± 1.10% vs. 16.16% ± 1.90%, *P* < 0.01; ∆% vBMD: 19.08% ± 3.52% vs. 9.26% ± 5.15%, *P* < 0.01) and also between the start of treatment and final evaluation (∆% AP-BMD: 61.23% ± 1.61% vs. 56.97% ± 1.45%, *P* < 0.01; ∆% vBMD: 36.69% ± 5.01% vs. 28.01% ± 5.76%, *P* < 0.01) (Fig. 2).

Simple regression analysis showed in group B a significant relationship between duration of calcium supplementation and ∆% change at final evaluation respect to pretherapy for AP-BMD (*r* = 0.41, *P* < 0.05) and vBMD (*r* = 0.40, *P* < 0.05) (Fig. 3).

Discussion

It is well known that BMD increase is age dependent (1) and about half of the adult PBM is accumulated during adolescent growth spurt (2) when dietary calcium requirements increase substantially (15). In females, the maximum increase in BMD at lumbar spine occurs between 11 and 14 yr and approaches its peak at age 16–17 yr (16, 17). The magnitude of PBM achieved during adolescence depends not only on genetic potential (race, sex, and heredity) (18, 19) but also on nutritional factors (calcium intake) (20, 21), physical activity (22, 23), pubertal calendar disruption (2), and body composition (24).

Regarding body composition, BMI sd score of females with CPP has been reported to be greater than that of controls before, during, and after GnRH therapy (25–27), as in our patients. However, in our patients, fat mass at final evaluation was not different from that in controls.

GnRHa treatment in patients with CPP is effective in decelerating the rates of linear growth and bone maturation, thus improving significantly final adult height and preserving genetic height potential (28), as in our patients. On the other hand, GnRHa treatment, stopping the progression of pubertal development, and reducing serum estradiol levels to prepubertal levels leads to a situation of hypoestrogenism, which may be accompanied by delayed skeletal maturation.
and deficient bone mineralization (4, 5, 29). In fact, estrogens have an important role in promoting normal bone maturation, accruing and maintaining BMD, and controlling bone turnover rate (30).

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).

In the present cohort of all patients, parameters studied (final TBMC, TBMD, AP-BMD L2-L4, and vBMD) were in the normal range, even if lower than in controls, but with no statistically significant difference. Therefore, GnRHa treatment in our patients with CPP does not seem to impair the achievement of a normal PBM, as previously reported (26, 32, 33). However, for the first time, we report the PBM at growth completion in patients treated with GnRHa and calcium. In these patients vBMD levels at stop of therapy and final evaluation were significantly higher than those treated with only GnRHa. Moreover, Δ% AP-BMD and Δ% vBMD between the beginning of treatment and final evaluation were also significantly higher in calcium-supplemented patients than those treated with only GnRHa, with a significant relationship with the duration of calcium supplementation.

The study has some limitations. The first is that the follow-up of bone mass is obtained by two different DXA machines. Replacement of DXA equipment was necessary in time as a result of upgrading according with the manufacturer. Absolute values of BMD using DXA differ between instruments; however, in literature, the rates of change calculated from serial measurements on different densitometers have been assumed to be comparable. Cross-calibration in general is considered to be the result of linear regression between the measurements obtained with two densitometers. This method is necessarily used in multicentric studies (26, 34, 35) or very long longitudinal studies as in our case (36). The second limitation is that, as well known, PBM is not achieved at final height but later in life (1). Therefore, the effect of precocious puberty and its treatment on PBM should be reevaluated later, at approximately 20–30 yr of age.

It is well known that adolescents often do not have a sufficient calcium intake (1, 37), and the mean calculated calcium intake in our noncalcium-supplemented patients and controls was lower than the recommended dietary allowances. On the contrary, calcium supplementation in patients of group B permitted to reach an average dietary calcium intake approximating the recommended dietary allowance and enhanced the rate of BMD increase.

On the other hand, calcium intake above the recommended dietary allowances is positively associated with bone mass in prepubertal (38, 39) and postpubertal (40, 41) females. Moreover, calcium supplementation in premenarcheal and perimenarcheal period, which appears to be the best time for bone calcium deposition (15, 42), seems to be important to reach a higher PBM and avoid the risk of postmenopausal osteoporosis (1).

Probably the majority of the general population does 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 (15, 43).

In conclusion, in our patients with CPP treated with long-term depot GnRHa, final bone densitometric parameters were in the normal range for age and sex. In the group of patients supplemented with calcium, calcium supplementation is effective in improving bone densitometric levels and may preserve better PBM achievement.

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