Longitudinal Follow-Up of Bone Density and Body Composition in Children with Precocious or Early Puberty before, during, and after Cessation of GnRH Agonist Therapy

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We studied bone mineral density (BMD), bone metabolism, and body composition in 47 children with central precocious puberty (n = 36) or early puberty (n = 11) before, during, and after cessation of GnRH agonist. Bone density and body composition were measured with dual energy x-ray absorptiometry and expressed as SD scores. Bone age and biochemical parameters of bone turnover were assessed. Measurements were performed at baseline, after 6 months, and on a yearly basis thereafter.

Mean lumbar spine BMD SD scores for chronological age were significantly higher than zero at baseline and decreased during treatment. Lumbar spine bone mineral apparent density and total body BMD did not differ from normal at baseline and showed no significant changes during treatment.

Contrast, BMD SD scores for bone age were significantly lower than zero at baseline and at cessation of therapy. Two years after therapy, bone mineral apparent density and BMD SD scores for bone age and chronological age did not differ from normal. Markers of bone turnover decreased during treatment, mainly in the first 6 months. Patients had increased percentage of fat and lean body mass at baseline. After an initial increase of percentage body fat during treatment, percentage body fat decreased and normalized within 1 yr after cessation of treatment.

Our longitudinal analysis suggests that peak bone mass or body composition will not be impaired in patients with precocious or early puberty after GnRH agonist therapy. (J Clin Endocrinol Metab 87: 506–512, 2002)

Subjects and Methods

Forty-seven patients (5 boys and 42 girls) were enrolled in the study. The mean age at start of GnRH-a treatment was 8.3 yr (range, 2.8–11.4 yr). At diagnosis, all patients had a history of increased growth velocity, girls had breast stage at least 2, boys had genital stage at least 2 and testes volume at least 4 ml, bone age was advanced more than 1 yr, and a GnRH-stimulated serum LH concentration was greater than 10 IU/liter. Thirty-one children had idiopathic CPP with start of puberty before the age of 9 yr for boys and before the age of 8 yr for girls. Five children had organic CPP (two meningomyelocele, one hydrocephalus, one optic glioma, and one craniopharyngioma). Idiopathic early puberty was found in 11 children, defined as appearance of pubertal signs between 8–10 yr of age for girls and between 9–11 yr of age for boys.

All patients were treated with depot leuprolide-acetate 3.75 mg (Lucrin Depot, Abbott Laboratories, Abbott Park, IL) given sc every 2 wk in the first month and every 4 wk thereafter.

Pubertal suppression was evaluated by clinical evaluation, by repeating GnRH stimulation test after 3 months, by measuring basal serum levels of LH, FSH, and E2 or T levels every 6 months of treatment. All children had complete suppression of LH and FSH during the GnRH test after 3 months of treatment (levels <5 IU/liter). All patients, except one boy and one girl, had complete suppression during therapy, with prepubertal basal sex steroid concentrations (E2 <50 pmol/liter; T <1
nmol/liter). In the boy and girl with incomplete suppression, GnRH-a dose was doubled, which resulted in complete suppression.

Anthropometry has assessment of biochemical parameters of bone turnover, and BMD and body composition measurements were performed at baseline, after 6 months and 1 yr, and then yearly. All assessments were continued after discontinuation of treatment on a yearly basis. Forty patients completed treatment, of whom 38 agreed to continue participation. Twenty patients (2 boys and 18 girls; 15 CPP and 5 early puberty) had complete follow-up measurements from start of GnRH-a therapy until 2 y after cessation of treatment. This subgroup will be described separately.

Height was measured with a Harpenden stadiometer and expressed as sd score (9). Pubertal stage was assessed according to Tanner (10). Bone age was scored by one investigator using an x-ray of the left hand according to the Greulich and Pyle method (11).

### Bone density and body composition

BMD of the lumbar spine (LS) and total body (TB) was measured by dual energy x-ray absorptiometry (DEXA) (DPXL/PED, Lunar Corp., Madison, WI). The LS is mainly composed of trabecular bone, whereas 80% of the TB bone consists of cortical bone (12). To correct for bone size, we calculated bone mineral apparent density (BMAD) of LS with the model BMADLS = BMDLS × 4/(π × width). This model has been validated by in vivo volumetric data obtained from magnetic resonance imaging of lumbar vertebrae (13). All children were measured by the same apparatus. Quality assurance was performed daily. The coefficient of variation has been reported to be 1.04% for spine BMD and 0.64% for TB BMD (14). Quality assurance was performed by TB DEXA. BMD, bone mineral content, lean tissue mass, and percentage body fat were compared with our Dutch age- and sex-matched reference values (n = 500; age, 4–20 yr) (15, 16). One girl was too young (2.8 yr) to compare her DEXA results with our normative data. Her data could be used to compare our Dutch age- and sex-matched reference values (n = 38) with our Dutch age- and sex-matched reference values (n = 38).

### Biochemical parameters

Blood samples were obtained for the assessment of calcium (Ca), anorganic phosphate, and 1,25-dihydroxyvitamin D. Furthermore, alkaline phosphatase (ALP) and procollagen type I C-terminal propeptide (PICP) were assessed as markers of bone formation, whereas carboxyterminal telopeptide of type I collagen (ICTP) and urinary hydroxyproline (OHP) concentration were measured as markers of bone resorption.

1,25-Dihydroxyvitamin D was measured by RIA of Immuno Diagnostics Systems (Boldon, UK). RIA kits (Orion Diagnostica, Espoo, Finland) were used for measurement of PICP and ICTP. ALP, phosphatase, PICP, and ICTP were expressed as sex- and age-matched sd scores using our own reference values. E2 and T were measured by RIA (Orion Diagnostica); LH and FSH by RIA (MedGenix Diagnostics, Fleurus, Belgium). The Ca/creatinine (Cr) ratio and OHP concentration, expressed as mmol/mol Cr (OHP/Cr), were assessed in the first morning void of urine. Written informed consent was obtained from the parents of the patients.

### Statistical analysis

One-sample t tests were used to compare the mean sd scores with the expected zero, which is the mean sd score of age- and sex-matched healthy controls. The within-patient change was tested using a paired t test. To test differences between patients with early puberty and precocious puberty, Mann-Whitney U tests were used. Pearson’s correlation coefficient was used to test the association between two variables with a skewed distribution. In view of multiple tests, the significance level was set at $P = 0.01$.

### Results

#### Bone density and body composition before treatment

Clinical baseline characteristics are reported in Table 1. The results of BMD and body composition before and during treatment for all patients are shown in Table 2. At baseline, mean BMDLS sd score was significantly higher than zero. BMADLS and BMDTB sd scores were above normal, but this did not reach significance. However, after correction for bone age instead of chronological age, BMDLS and BMDTB sd scores corrected for bone age were significantly lower than zero. BMADLS sd score corrected for bone age was normal.

Height, bone mineral content, percentage fat, lean body mass (LBM), and body mass index (BMI) sd scores (all sd scores for chronological age) were significantly increased at baseline.

Forty patients completed treatment; their mean treatment period was 2.7 yr (range, 1.4–5.4 yr). After an initial increase,
BMD_{LS} and BMD_{TB}, corrected for chronological age, tended to decrease (Table 2). BMAD_{LS} showed the same trend, but these changes were not significant. However, when adjusted for bone age, BMD_{TB} sd scores for bone age increased significantly during treatment. As for chronological age, no significant changes in BMAD_{LS} corrected for bone age were found during treatment. BMD_{LS} sd scores for bone age also did not change during treatment.

Percentage body fat sd scores increased, and LBM sd scores decreased significantly (Table 2). Height sd scores decreased; after 1 yr of treatment height sd scores did not differ significantly from zero any more. BMI sd scores increased significantly during treatment.

Results of 20 patients followed from start to 2 yr after cessation of therapy

Figure 1 shows bone density and body composition before, at cessation of GnRH-a treatment, and 2 yr after cessation of treatment in 20 patients who were followed for this entire period. For this particular subgroup, mean (sd) age and bone age at start was 8.7 (1.1) and 11.3 (1.24) yr, respectively. Mean (sd) age at cessation of therapy was 11.3 (0.8) yr. Mean (sd) bone age was 12.4 (0.7) yr at cessation of therapy, and 14.6 (1.0) yr at 2 yr after cessation of therapy. Mean treatment period for this subgroup was 2.6 yr (range, 1.4–4.1 yr).

At baseline, BMD_{LS} sd score adjusted for chronological age was 0.54 (sd = 1.15; \( P = 0.06 \)). BMD_{LS} sd scores for chro-

![Fig. 1. BMD adjusted for chronological age (top) and bone age (bottom) before start, at cessation, and after cessation of GnRH-a therapy in children with precocious and early puberty [mean (SEM)]. * \( P < 0.01 \), \( b P < 0.001 \) for the comparison of the mean sd score with zero; *, \( P < 0.01 \), **, \( P < 0.001 \) compared to stop.]
nological age decreased significantly during treatment, whereas BMD\textsubscript{TB} and BMAD\textsubscript{LS} \textit{sd} scores showed no significant change. After cessation of therapy, no significant changes in bone density were found (Fig. 1, top).

After correction for bone age (Fig. 1, bottom), only BMD\textsubscript{TB} showed a significant increase, whereas BMAD\textsubscript{LS} and BMD\textsubscript{LS} remained significantly lower than zero at cessation of treatment. After cessation of GnRH-a therapy, all three bone density parameters slightly increased compared with stop of therapy. After 2 yr, none of the bone density parameters differed from zero any more.

The mean absolute change between start and cessation of GnRH-a therapy was $0.09 \text{ g/cm}^2$ for BMD\textsubscript{LS} and BMD\textsubscript{TB} and $0.01 \text{ g/cm}^3$ for BMAD. None of the children had an absolute decrease in BMD during therapy. Most children showed an absolute increase in BMAD as well, but in three children a decrease in BMAD (0.01–0.02 g/cm\textsuperscript{3}) was found. In the 2 yr after cessation of therapy, BMD and BMAD showed an absolute increase in all children.

LBM decreased significantly during treatment, and percentage body fat increased (Fig. 2). After cessation of treatment, LBM and height showed a further decrease, whereas percentage body fat decreased to pretreatment values. Although BMI decreased significantly after treatment was stopped, it remained significantly higher than zero after treatment. Two years after cessation of therapy, LBM, percentage body fat, and height \textit{sd} scores did not differ significantly from zero.

\textit{Biochemical parameters}

Table 3 shows the results of biochemical parameters at baseline, during treatment, and after cessation of treatment. Only baseline and 6-month data are given, because the substantial changes were found in this period. During GnRH treatment, serum Ca was normal and remained stable. At baseline, ICTP \textit{sd} score was significantly higher than zero. PICP \textit{sd} score was also increased, but in a lesser degree ($P = 0.03$). Mean phosphate \textit{sd} scores, ALP \textit{sd} scores, 1,25-dihydroxyvitamin D, and Ca/Cr ratio were normal. Urine OHP concentration was in the high reference range. ALP, ICTP, and PICP \textit{sd} scores and urine Ca/Cr ratio decreased significantly during treatment, mainly in the first 6 months, and stabilized thereafter. Phosphate \textit{sd} scores showed a slight decrease. Urinary OHP concentration showed an ongoing decrease, whereas urine Ca/Cr ratio did not change.

\textit{Precocious puberty vs. early puberty}

The mean treatment period was 2.8 yr in the CPP patients and 2.2 yr in patients with early puberty. Patients with CPP had higher height and LBM \textit{sd} scores than children with early puberty at baseline and at cessation of treatment. No differences in BMI and percentage body fat were found. At baseline, BMD and BMAD for chronological age and bone age did not differ. BMD\textsubscript{TB} and BMD\textsubscript{LS} for chronological age were significantly higher in the CPP group at cessation of therapy. BMAD and BMD for bone age did not differ at cessation of treatment.

\textit{Correlations}

The changes ($\Delta$) between values at cessation of treatment and at start of treatment were calculated. $\Delta$Height and $\Delta$LBM \textit{sd} scores were positively correlated with $\Delta$BMD\textsubscript{LS} ($r = 0.46$, $P = 0.007$; $r = 0.61$, $P < 0.001$, respectively), but not with

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**Fig. 2.** Body composition and height adjusted for chronological age (mean (SEM)) at start, at cessation, and after cessation of GnRH-a therapy in children with precocious puberty. *, $P < 0.01$; **, $P < 0.001$ for the comparison of the mean \textit{sd} score with zero; *, $P < 0.01$, **, $P < 0.001$ compared to stop.
Biochemical parameters at baseline and after cessation of GnRH agonist treatment in children with precocious puberty [mean (SEM)]

<table>
<thead>
<tr>
<th></th>
<th>Before and during GnRH-a</th>
<th>After cessation of GnRH-a</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 months</td>
</tr>
<tr>
<td>No.</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>Ca (mmol/liter)</td>
<td>2.42 (0.02)</td>
<td>2.42 (0.02)</td>
</tr>
<tr>
<td>Phosphate SD score</td>
<td>−0.07 (0.11)</td>
<td>−0.23 (0.12)</td>
</tr>
<tr>
<td>ALP SD score</td>
<td>0.10 (0.19)</td>
<td>−1.05 (0.15)**</td>
</tr>
<tr>
<td>PICP SD score</td>
<td>0.57 (0.25)</td>
<td>−0.74 (0.21)**</td>
</tr>
<tr>
<td>ICTP SD score</td>
<td>1.78 (0.14)b</td>
<td>0.26 (0.14)**</td>
</tr>
<tr>
<td>1,25-dihydroxyvitamin D (pmol/liter)</td>
<td>132.1 (7.0)</td>
<td>125.1 (7.1)</td>
</tr>
<tr>
<td>Urine Ca/Cr ratio</td>
<td>0.20 (0.02)</td>
<td>0.40 (0.04)**</td>
</tr>
<tr>
<td>Urine OHP/Cr ratio (mmol/mol)</td>
<td>132 (9)</td>
<td>104 (16)**</td>
</tr>
</tbody>
</table>

* P < 0.01, b P < 0.001 for the comparison of the mean SD score with zero; a, P < 0.01, **, P < 0.001 compared with baseline; 1 P < 0.01, 2 P < 0.001 compared to stop.

BMAD$_{LS}$ or BMD$_{TB}$, ΔBMI SD score was not significantly correlated with ΔBMD or ΔBMAD SD scores. Changes in parameters of bone turnover showed correlation with neither Δheight SD scores nor changes in BMD or BMAD SD scores.

**Discussion**

The present study showed increased BMD$_{LS}$ for chronological age in children with precocious or early puberty. During GnRH-a treatment, BMD and BMAD of lumbar spine and total body BMD for chronological age decreased, after an initial increase, to the normal range. However, after correction for bone age, BMD$_{LS}$ and BMD$_{TB}$ were reduced. Using BMAD$_{LS}$ (volumetric or apparent BMD) corrected for bone age normal bone density before, during, and after cessation of treatment was found.

Sex steroids, and especially estrogens, are very important in the acquisition of bone mass. This became more clear when two new syndromes were described, each representing a different endocrine basis of the full PBM syndrome: aromatase deficiency (17) and a male with ER defect (18) had BMADLS or BMD$_{TB}$. BMD also increases in healthy prepubertal children who are not exposed to high levels of sex steroids. This might explain that during gonadal suppression in our CPP patients BMD continued to increase.

Heger et al. (19) studied patients after cessation of GnRH-a therapy at final height. These young women had normal bone density of LS and femoral neck. Most studies found a BMD high for chronological age, but appropriate for bone age (19–21), before start of therapy. The described effects of GnRH-a on bone are still controversial. No change during 2 yr of treatment has been reported (20), as well as a decrease in trabecular bone density during GnRH-a therapy, whereas cortical bone of the radius did not change (22). Saggese et al. (23) reported a reduction in BMD in cortical bone of the radius within 6 months. Most studies were performed in rather small patient groups varying from 10–13 children. Additionally, differences in reference values and differences in site, which have been measured, and differences in timing of start of treatment may have contributed to some of the discrepancy with our results. Furthermore, our study also included children with early puberty. Children with CPP were taller but did not have higher BMD than children with early puberty, at baseline. Despite a somewhat longer treatment in CPP, BMD was even somewhat higher in CPP at cessation of therapy. This difference disappeared after correction for bone size by calculating BMAD. Bertelloni et al. (24) stated that PBM was not impaired in girls treated with GnRH-a. Because PBM of LS was measured at the chronological age of 13.4 yr, this might have been too early. Bone mineral accrual continues in the postpubertal years after linear growth has ceased. Furthermore, the age that PBM is reached appeared to be site-dependent (25). Our patients had mean age of 13.4 yr and bone age of 14.7 yr at their last visit. Theoretically, children with precocious puberty might attain their PBM at an earlier chronological age, because of their advanced bone maturation. However, all patients showed an ongoing increase in BMD$_{LS}$ after cessation of therapy. So, we cannot draw any final conclusions yet regarding PBM. Nonetheless, our findings, e.g. normal bone mass and bone turnover 2 yr after cessation of therapy, do not suggest that PBM will be impaired in children with a history of GnRH-a therapy.

We found that the ICTP was significantly higher and PICP was slightly higher than normal before the start of treatment.
During treatment, bone turnover decreased mainly in the first 6 months of treatment and stabilized thereafter. Other authors found similar results. Antoniazzi et al. (22) showed that patients with CPP had pubertal osteocalcin levels that decreased during treatment. Hertel et al. (26) reported that girls with CPP had normal PICP levels that decreased within 2 months after initiation of GnRH-a and remained below baseline values. Therefore, markers of bone resorption as well as markers of bone formation decrease during GnRH-a therapy. However, biochemical markers are not specific for bone modeling or remodeling. Additionally, PICP, ICTP, and ALP are not bone-specific. Thus, changes in biochemical markers may reflect changes in growth as well as changes in bone mineralization. Two years after cessation of therapy, the markers of bone turnover were in the normal range for bone age.

Obesity is a common problem in children with precocious puberty (6, 27, 28). Indeed, in the present study, percentage body fat was increased at baseline. At cessation of therapy, percentage body fat was significantly higher than normal, and it normalized thereafter. After an initial aggravation of adiposity, no prolonged negative effects on percentage body fat were found. In concordance with our study, two other studies showed increased BMI at the start but no change in BMI during treatment (6, 19). BMI cannot distinguish between LBM and fat mass; BMI will not change when LBM decreases and fat mass increases as occurred in our study. Therefore, DEXA is preferred to evaluate body composition.

The decreased GH and IGF-I levels that have been reported during GnRH-a therapy (27, 29, 30) might play a role in the incremental rate of fat mass and decrease in BMI. Kamp et al. (27) showed that GH levels in GnRH-a-treated children with CPP were inversely related to BMI. GH-deficient patients have decreased bone density, increased fat mass, and decreased LBM, which improved during GH replacement therapy (31). Besides a decrease in estrogens and GH levels, the decrease in muscle mass during GnRH-a therapy may also affect bone mineralization.

In conclusion, children with precocious and early puberty had normal total body BMD and increased BMD_{LS} for chronological age. Only BMD_{LS} decreased significantly during GnRH-a therapy. In contrast, BMD corrected for bone age was significantly lower than normal. Using BMAD_{LS} (volumetric or apparent BMD) corrected for chronological age or bone age, normal bone density before, during, and after cessation of treatment was found. Two years after cessation of therapy, bone density for bone age and chronological age did not differ from normal, and markers of bone turnover adjusted for bone age were normal. Thus, this study suggests that PBM will not be impaired in children with a history of GnRH-a therapy. However, our patients did not reach their PBM yet, so further follow-up is still needed. After an initial aggravation of adiposity during treatment, their percentage body fat decreased to normal values after treatment was stopped.

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References

Asymptomatic Primary Hyperparathyroidism
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Bethesda, Maryland
April 8–9, 2002

A 1990 NIH Consensus Development Conference on Asymptomatic Primary Hyperparathyroidism, sponsored by NIDDK, explored the causes and effects of this disease, developing a consensus on how to treat it. Together with a research agenda, the consensus statement provided landmarks for basic and clinical approaches to the disease. Since then, many new advances have occurred, in many instances providing new approaches to treatment.

Now in 2002, this workshop will attempt to define the current state of the art by focusing on the latest basic and clinical advances that have been made in our understanding of this disease, with an eye on identifying new directions for the 21st century. Posters are welcome.

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