Bone Mineral Density and Bone Metabolic Markers in Children with Hyperthyroidism Before and During Treatment

Yumi Asakura, Masanori Adachi and Katsuhiko Tachibana

Department of Endocrinology and Metabolism, Kanagawa Children's Medical Center, Kanagawa, Japan

Abstract. Increased bone turnover and loss of bone mass in adult patients with hyperthyroidism is well documented, but bone manifestations in children with hyperthyroidism are poorly understood. We evaluated cortical bone mineral density (BMD) and bone metabolic markers in two boys and five girls, aged 5.7–16 yrs (12.2 ± 3.7 yrs) with hyperthyroidism before and during treatment. BMD (ΣGS/D) was assessed by computed X-ray densitometry in the second metacarpal bone of the left hand. The %BMD (percentage for expected ΣGS/D of age- and sex-matched standard) before treatment was 90.5 ± 8.5% (M ± SD), which was lower than normal children (p<0.05). After 6 months of treatment, %BMD increased to 95.9 ± 9.5%, showing partial restoration. Basal levels of urinary N-telopeptides of collagen type 1 (NTx) before treatment were markedly high, but decreased rapidly during treatment towards the normal range. We also found a transient rise in NTx on day 4 of treatment before the decrease. Basal levels of serum bone alkaline phosphatase were as high as the upper limits of the reference ranges in pubertal children, slightly decreasing for the first two weeks of treatment, increasing transiently for a few months and declining thereafter. We demonstrated decreased BMD in children with hyperthyroidism for the first time. Bone resorption rapidly ameliorated, and accelerated bone formation persisted for a few months after normalization of thyroid function, while BMD improved at 6 months’ evaluation, as reported in adult patients. We also found a transient increase in bone resorption markers and initial suppression of bone formation markers before the amelioration. A longer period of follow up with more patients is needed to clarify how the mechanism of bone metabolism is affected by thyroid function.

Key words: bone mineral density, computed X-ray densitometry, hyperthyroidism, urinary N-telopeptides of collagen type 1, bone alkaline phosphatase

Introduction

Abnormal bone metabolism in adult patients with hyperthyroidism is well documented and characterized by increases in both osteoclastic and osteoblastic activities, with a predominance of bone resorption resulting in increased levels of bone turnover markers and decreased bone mass (1~4). Since the majority of bone mass acquisition occurs during childhood and adolescence, decreased acquisition during these critical periods could be a risk factor for osteoporosis in adulthood, which is a major public health problem. However, only a little information is available regarding the
effect of hyperthyroidism and its treatment on bone mineral density and metabolism in children.

Serum bone alkaline phosphatase (B-ALP), determined by an enzyme-immunoassay (EIA) (5), and urinary N-telopeptides of collagen type 1 (NTx) determined by an enzyme-linked immunosorbent assay (ELISA) (6), have been introduced as formation and resorption bone markers, respectively. In the present study those markers were applied to clarify bone metabolism in children with hyperthyroidism and its recovery period after attainment of euthyroidism. We also evaluated cortical BMD (ΣGS/D) as assessed by computed X-ray densitometry in the second metacarpal bone along with the two bone markers.

Subjects and Methods

Patients
Two boys and five girls, aged 5.7–16 yrs. (12.2 ± 3.7 yrs) with recent onset of hyperthyroidism were enrolled. All patients were Japanese and diagnosed as having Graves’ disease. In all subjects, serum thyroid hormone levels were elevated and TSH suppressed (<0.03 µIU/ml). All subjects were treated with thiamazole, except one who was changed to propylthiouracil because of arthralgia. FT4 levels returned to normal within 2 months of treatment in all children, except the one who changed medication.

Methods
Morning samples of venous blood were drawn from patients for measurement of B-ALP and thyroid hormone. On the same morning, urine samples were collected as the second void of the day for the measurement of creatinine and NTx. All samples were stored at –20°C until assayed.

Serum B-ALP levels were measured with an enzyme-immunoassay (Osteolink BAP). Since the mean values of B-ALP in normal children using this method have not been published, we converted reported normative data by IRMA expressed as mass concentration (µg/L) (9) into a value of activity concentration (U/L) by EIA using the formulas y=1.03x+0.22 from IRMA into EIA (5), and y=1.33x+3.11 from mass concentration (µg/L) into a value of activity concentration (U/L) (10). Urinary NTx levels were measured using an enzyme-linked immunosorbent assay (Osteomark) and assay values were corrected for urinary dilution by urinary creatinine concentration. Results were expressed as nmol of bone collagen equivalent (BCE) per mmol creatinine (mmol Cr) and compared with the mean values for normal children (11, 12). Urinary calcium/creatinine excretion ratios (u mgCa/mgCr) were evaluated to assess calcium metabolism.

Hand bone X-rays were taken with an aluminum step-wedge ruler before and 6 months after starting therapy. BMD was measured with a quantitative analyzing system (Bonalyzer, Teijin, Tokyo) integrating the densitometric pattern area per bone width (ΣGS/D) of the second metacarpal bone in the left hand. This is dubbed the computed X-ray densitometry (CXD) method, which is an improved micro-densitometry method (7). Standard values for normal Japanese children were reported as cross-sectional data by age (8). Under the assumption that the standard values should increase linearly between two successive years we interpolated the findings of two successive years and calculated standard values by month for each gender. The findings from our patients were compared with these standards and a BMD-SD score and %BMD (percentage for expected ΣGS/D of age- and sex-matched standard) were evaluated. Bone age was estimated using the Greulich & Pyle method.

Statistical analysis
All values are expressed as the mean ± SD unless otherwise noted. Mean values of patients and normal standards were compared using Student’s t-test. Student’s t-test was also used for paired samples of BMD before and 6 months after starting treatment. For repeated measurements of
serum B-ALP and urinary NTx, one-way or repeated measures ANOVA was applied. P<0.05 was considered significant.

**Results**

**Bone mineral density**

BMD-SD score and %BMD before treatment in the 7 children (2 male, 5 female, CA 12.2 ± 3.7 years) were evaluated. The values were −0.92 ± 0.50 and 90.5 ± 8.5%, respectively, which were lower than the normal standard for their chronological age (p<0.01, p<0.05) (Table 1). The mean for their bone age was 13.3 ± 3.3 years, slightly accelerated in comparison with chronological age. When bone age was substituted for chronological age, BMD-SD score and %BMD became −1.64 ± 0.86 and 84.9 ± 7.2%, respectively, and the reduction in BMD before treatment was more apparent (p<0.01, p<0.01) (Table 1).

Evaluation of BMD before and 6 months after starting therapy is shown in Table 2. The mean value of BMD increased significantly from 1.83 ± 0.48 before treatment to 1.97 ± 0.44 after 6 months (p<0.01). As BMD in normal children is also expected to increase at an interval of 6 months, changes in BMD were evaluated by changes in %BMD before treatment and after 6 months of therapy. The mean %BMD also increased significantly from 90.5 ± 8.5 % to 95.9 ± 9.5% after therapy (p<0.05). Anti-thyroid treatment resulted in an increase in BMD, which was still 5% lower than the standard after the 6 month period of follow-up, although this difference was not significant.

**Urinary NTx and serum B-ALP in untreated children**

The basal NTx and B-ALP levels of the 7 children before treatment were 1144.7 ± 495.8

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Before therapy</th>
<th>6 months after therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13.7</td>
<td>f</td>
<td>4</td>
<td>1.785 79.3</td>
</tr>
<tr>
<td>B</td>
<td>9.3</td>
<td>f</td>
<td>1</td>
<td>1.510 96.2</td>
</tr>
<tr>
<td>C</td>
<td>11.4</td>
<td>f</td>
<td>1</td>
<td>1.590 85.5</td>
</tr>
<tr>
<td>D</td>
<td>5.7</td>
<td>f</td>
<td>1</td>
<td>1.090 81.9</td>
</tr>
<tr>
<td>E</td>
<td>14.4</td>
<td>f</td>
<td>4</td>
<td>2.335 99.8</td>
</tr>
<tr>
<td>F</td>
<td>16</td>
<td>m</td>
<td>4</td>
<td>2.420 100.0</td>
</tr>
<tr>
<td>G</td>
<td>15</td>
<td>m</td>
<td>4</td>
<td>2.045 90.7</td>
</tr>
</tbody>
</table>

| M ± SD | 12.2 ± 3.7 | 1.83 ± 0.48 | 90.5 ± 8.5 | 1.97 ± 0.44<sup>a</sup> <sup>c</sup> | 95.9 ± 9.5<sup>c</sup> |

%BMD/CA; percentage for expected BMD of sex- and age-matched standard. a; p<0.01, b; p<0.05, compared with before therapy using the paired Student’s t-test. c; p<0.05, ns; not significant, compared with sex- and age-matched standard using the Student’s t-test.
nmol BCE/mmolCr (nMBCE/mMCr) and 127.3 U/l, respectively. In a report on standard values of NTx in relation to pubertal stages, maximum excretion of NTx was observed at the beginning of adolescence and the values were 247.5±57.2 (M±SEM) nMBCE/mMCr for boys and 353.5±23.2 nMBCE/mMCr for girls (12). Standard values of B-ALP were reported as being highest at adolescence and the mean values were approximately 140 U/l for girls at the age of 11–12 and for boys at the age of 13–14, as calculated by regression equations (5, 9, 10). Basal mean NTx of our 7 children was three- to four-fold higher than the mean of normal pubertal children (11, 12) and basal mean B-ALP was as high as normal pubertal children (9).

**Urinary NTx and serum B-ALP in treated children**

The bone markers of 5 patients were analyzed at 0, 4 days, 1, 2, 3, 4, 8, 12 and 24 weeks after treatment. Urinary NTx fell rapidly in all subjects after a small rise on day 4. The mean value of NTx increased from 1108.2±447.8 to 1689.3±1264.5 nMBCE/mMCr on day 4, but the difference was not significant (Fig. 2). The decline in urinary NTx after day 4 became significant by 4 weeks of therapy, as analyzed with repeated ANOVA (p<0.01). In comparison with the basal NTx, the mean NTx after therapy became significantly lower after 12 weeks treatment (one-way ANOVA; p<0.05). In contrast, B-ALP decreased significantly for the first 2 weeks (repeated ANOVA; p<0.05), and increased markedly thereafter, reaching a peak after around 8 weeks of therapy (Fig. 3). The peak of B-ALP at 8 weeks was significantly higher than basal B-ALP (one-way ANOVA; p<0.05). After the peak, B-ALP decreased gradually and reached levels similar to before treatment after 6 months of follow-up.

**Urinary excretion of calcium in treated children**

Urinary Ca/Cr was highest at the time of peak NTx (day 6±3.7), then declined markedly and became lowest at the time of peak B-ALP (day 72±21.7) (Fig. 4).

**Discussion**

This is the first published study that has shown bone manifestations in children with hyperthyroidism before and during treatment.
using the CXD method for evaluation of BMD, NTx and B-ALP as bone metabolic markers.

Bone loss in adult patients with hyperthyroidism has been documented and it has been reported that successful treatment of hyperthyroidism produces an increase in BMD, as assessed by dual energy X-ray densitometry (DEXA) (2, 3, 13), with an improvement in cortical bone striations (4). As bone morphometric studies have demonstrated enhanced osteoclastic bone resorption, especially in cortical bone in patients with hyperthyroidism, cortical BMD assessed by CXD could also be a useful method to clarify bone manifestations of patients.

In the present study, the mean value of BMD by CXD in untreated children was 10% less than expected and this reduction was partially restored after attainment of the euthyroid state. Assessments of BMD changes in adolescence are cumbersome, because BMD increases with age and pubertal development. Even though we compared %BMD, which is the percentage for expected ΣGS/D of age- and sex-matched standards, to eliminate the influence of age, it was difficult to eliminate the effects of advances in pubertal development during the follow up. Two of the patients showed advances in the pubertal stage during the 6 months of follow up (cases B and C), while the other 5 did not. All showed increases in %BMD, but pubertal advance could not explain all of these increases.

The recovery after the 6 months period of follow-up seemed incomplete, with a 5% deficit compared to mean values of BMD by CXD in normal children, although the difference was not significant. Similar incomplete reversibility in the trabecular bone of adult patients after 18 months of therapy has been reported (3).

Bone resorptive markers, such as type I collagen C-terminal peptide (14), urinary deoxypyridinoline and pyridinoline (3, 15, 16), increase in patients with hyperthyroidism. Urinary NTx is also one of the most sensitive markers of mature bone collagen degradation (11), and a marked increase by 8-fold above the normal mean during the hyperthyroid phase in adult patients was recently reported (17). The NTx levels of untreated children in the present study were three- to four-fold higher than the upper limits of the reference range in normal pubertal children, and the B-ALP levels before treatment were as high as pubertal children. The discrepancies in the degree of increases between the two markers in this study, NTx and B-ALP, suggest a predominance of bone resorption in hyperthyroidism which would result in bone mass loss in untreated patients. The same kinds of
discrepancies have been reported with different markers in adult patients (16, 17).

There was a large and prompt reduction in NTx during the first month of treatment, as reported recently by Pantazi et al. (17), suggesting activated bone absorption was rapidly suppressed following anti-thyroid treatment. A transient rise in mean NTx secretion was seen on day 4 and the same transient rises in urinary pyridinoline and deoxypyridinoline excretion, which are known as bone degeneration markers, were reported by MacLeod et al. (15). The reason for this increase is unclear, but may be related to very rapid withdrawal from thyroid hormone excess. In contrast, mean B-ALP slightly decreased for the first two weeks and increased thereafter, reaching a peak after approximately 2 months of treatment. Increases in bone formation markers, including B-ALP, have been demonstrated in several studies and peaks were identified after between 1 and 3 months of treatment (3, 4, 13, 16, 17). However, an initial decrease before the increase has not been identified previously. In previous studies, bone markers were measured with longer intervals and more frequent measurements may be necessary to detect the slight suppression phase in B-ALP. The exact reasons for the transient rise in NTx and the initial suppression of B-ALP are unclear. However, the initial suppression of B-ALP and its subsequent increase might be steps in the normalization of osteoblast function caused by amelioration of excessive thyroid hormone. Since it was reported that the activation of osteoclasts is mediated by osteoblasts (19), which have nuclear thyroid hormone receptors, there could be some interactions between the two kinds of osteocytes, leading to the first transient rise in NTx, although this could not be clarified in the present study. The rapid decrease in NTx after a small rise could also be due to reduced osteoclast activity mediated by osteoblast function. The persistence of the B-ALP concentrations following treatment are indicative of the continuing osteoblastic phase of remodeling cycles and predominant bone formation which persists for several months after the normalization of bone resorption. This subsequent predominant bone formation after predominant resorption appears to be important for the improvement of abnormal bone manifestations. A reduction in serum B-ALP concentrations towards the end of this study could be consistent with completion of this restorative phase. These findings suggested that the main changes in bone restoration occurred in the first 6 months of successful treatment, and that these changes may be insufficient to normalize BMD. We need a longer period of follow up to clarify BMD restoration after 6 months of treatment to assess the final prognosis. However, intensive supportive therapy for bone restoration in the first several months along with anti-thyroid treatment may be useful for better prognosis.

After hyperthyroidism treatment, serum calcium sometimes falls even though there is no damage to the parathyroid glands and this hypocalcemia is ascribed to the healing process of bone manifestations. Mosekilde et al. studied the effect of anti-thyroid treatment on calcium and phosphorus metabolism and demonstrated a decrease in urinary calcium excretion during the decline of urinary hydroxyproline and an increase in ALP, suggesting decreased bone resorption and increased bone formation with calcium deposition to bone after anti-thyroid treatment (18). Changes in calcium and phosphorus metabolism with other parameters of bone metabolism were reported by Pantazi et al., and the role of PTH contributing an anabolic effect on bone metabolism was discussed (17). Hypocalcemia was not observed in our patients, but urinary calcium secretion was markedly reduced during the predominant bone formation period. This may be due to rapid skeletal uptake of calcium. Intentional intake of calcium or vitamin D supplements during the treatment especially for the first 6 months of the active bone formation period, could be one of the potent recommendations to supply such demands.

A longer period of study with more subjects should be designed to clarify the final extent of
improvement, the potential benefits of supportive therapy for bone and also to address the mechanism of bone formation and resorption mediated by the thyroid hormone.

In conclusion, the present study showed cortical bone mass loss in children with hyperthyroidism and its partial restoration during therapy. This recovery phase was characterized by elevated levels of bone formation markers and reduced bone resorption markers and was focused in the first 6 months of effective treatment.

References


17. Pantazi H, Papapetrou PD. Changes in parameters of bone and mineral metabolism during therapy
