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# Bone mass and metabolism in thalassemic children and adolescents treated with different iron-chelating drugs

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**Abstract** We evaluated bone mineral density (BMD) and bone turnover in 22 homozygous prepubertal beta-thalassemic patients treated with desferrioxamine. Ten patients underwent treatment with desferrioxamine for the whole study period, while 12 patients stopped desferrioxamine and were then treated with deferiprone (L1). Lumbar and femoral BMD and bone metabolism markers were examined at baseline and after 1 and 3 years of follow up. All patients were prepubertal at baseline and they all became pubertal over the 3 years of follow up. At baseline, the mean lumbar Z score value was $-2.048$ SD $\pm$ 0.75; the Z score was less than $-2$ SD in 13 children, within $-1$ and $-2$ SD in 6, and within 0 and $-1$ SD in only 3 subjects. A significant BMD increase ($P < 0.0001$) was observed at both the lumbar (+8.466%/year) and the femoral level (average of +3.46%/year at neck and +5.83%/year at the intertrochanteric region) after 3 years, without any significant difference being shown between patients treated with desferrioxamine and those treated with L1. The mean Z score SD values increased to $-1.957 \pm 0.975$ at 1 year (not significantly different from baseline) and to $-1.864 \pm 1.221$ at 3 year follow up ($P < 0.05$ vs baseline); an increase in bone turnover was also observed. These findings show that low BMD, a hallmark of beta-thalassemia, improves significantly when puberty begins; this increase involves different skeletal sites, regardless of pharmacological treatment with different iron-chelating drugs.

**Key words** bone mineral density · deferiprone · desferrioxamine · dual X-ray absorptiometry · thalassemia

## Introduction

Homozygous beta-thalassemia is a severe hemolytic disorder associated with a variety of skeletal abnormalities, which include growth failure, fractures, and osteopenia [1–5].

Several causes can be proposed for the osteopenia, such as delayed puberty and hypogonadism [6–8]; expansion of the medullar cavity due to bone marrow hyperplasia, and the subsequent thinning of the adjacent bone [9]; slow bone formation and reduced mineral deposition due to exogenous hemochromatosis and deposition of iron in the osteoblasts [3,10,11]; vitamin D deficiency due to chronic liver disease [3,12,13]; treatment with iron chelators [14]; and concomitant secondary endocrinopathies, such as growth hormone (GH) deficiency, diabetes mellitus, and hypo- and hyperparathyroidism [1,15,16].

Thalassemia used to be a potentially fatal condition in childhood, but, due to therapeutic improvements, patients have now achieved a longer life expectancy, and can now take part in daily activities, and are thus exposed to greater risk of fracture due to osteoporosis.

Therefore, the aim of this study was to evaluate bone mass and metabolism behavior in homozygous beta-thalassemia patients from the prepubertal to pubertal stage, and to investigate the effects of iron chelation on bone metabolism and on the development of thalassemic osteopathy.

## Subjects and methods

With the approval of our Institutional Ethics Committee, we evaluated 22 homozygous beta-thalassemic patients (13 boys and 9 girls) at the prepubertal stage (mean age at baseline, 11.4 $\pm$ 1.78 years; median, 11.2 years; range, 8.1–14.9 years) and after 1 and 3 years of follow up.

All patients were transfused at regular intervals to keep hemoglobin levels up to at least 12 g/dl; at the beginning of the study all patients had already been treated for a long time with desferrioxamine (daily s.c.

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**Table 1.** Sex, age, height, weight, and ferritin levels of the examined subjects at baseline

| Patient no. | Sex | Age (years) | Height (cm) | Weight (kg) | Ferritin level (ng/dl) |
|---|---|---|---|---|---|
| 1 | M | 10 | 138 | 26.5 | 1705 |
| 2 | M | 8 | 133 | 30 | 1234 |
| 3 | F | 12 | 136 | 34 | 1189 |
| 4 | M | 10 | 130 | 26.5 | 1175 |
| 5 | F | 9 | 126 | 25 | 1231 |
| 6 | M | 10 | 132 | 26.5 | 1362 |
| 7 | M | 9 | 131 | 22.5 | 493 |
| 8 | F | 11 | 141 | 24.5 | 1489 |
| 9 | M | 14 | 134 | 28.5 | 1582 |
| 10 | M | 12 | 124 | 21.5 | 1870 |
| 11 | F | 13 | 131 | 33.5 | 1657 |
| 12 | F | 11 | 132 | 29.5 | 1601 |
| 13 | F | 11 | 126 | 26.5 | 1578 |
| 14 | M | 10 | 124 | 21 | 1918 |
| 15 | M | 13 | 139 | 32.5 | 1800 |
| 16 | F | 11 | 134 | 35 | 1632 |
| 17 | M | 11 | 135 | 34 | 2097 |
| 18 | M | 11 | 132 | 27.5 | 1906 |
| 19 | F | 14 | 140 | 33 | 913 |
| 20 | F | 8 | 124 | 27 | 1406 |
| 21 | M | 14 | 152 | 35.2 | 1759 |
| 22 | M | 14 | 150 | 45 | 1691 |

infusions of 30–40 mg/kg; compliance, 85%). After the baseline measurements, the patients were randomly divided into two groups: 10 patients continued treatment with desferrioxamine at the same dose, while 12 patients started treatment with deferiprone (L1) at 75 mg/kg four times daily for 3 days per week.

Sex, age, height, weight, and ferritin levels (ferritin was always kept below 2500 ng/dl in all subjects) at baseline are shown in Table 1.

All subjects were free of diet restrictions and none were affected by liver, thyroid, kidney, or heart diseases, GH deficiency, or diabetes mellitus. Six boys and three girls had been splenectomized 2 to 7 years earlier.

We evaluated fasting serum calcium, magnesium, and phosphate levels; bone alkaline phosphatase (BAP); and urinary deoxypyridinoline crosslinks. Bone alkaline phosphatase (BAP) was measured by immunoradio metric assay (IRMA; interassay coefficient of variation [CV], 3.4%; intraassay CV, 3.6%) and urinary crosslinks levels were measured by enzyme-linked immunosorbent assay (ELISA; interassay CV, 4.6%; intraassay CV, 4.3%).

Bone mineral density (BMD) was measured at the lumbar spine (L1-L4) and right hip. BMD was evaluated by a dual-energy X-ray absorptiometry (DXA) technique, with a QDR 1000 densitometer (Hologic, Waltham, MA, USA). The results were expressed as absolute values (g/cm$^2$) at femoral sites and also as a Z Score (difference in SD from healthy age- and sex-matched subjects) only at the lumbar spine, because the normal range for healthy age-matched children was not available at the femoral level. The reproducibility of the DXA technique at the lumbar spine is 0.5% in vitro, and 1% in vivo in adults.

**Table 2.** Mean BMD values at lumbar level (results expressed as Z scores) at baseline and after 1 and 3 years' follow up

| | Mean value (SD) | $P$ vs baseline |
|---|---|---|
| Baseline | −2.048 (0.75) | — |
| 1 year | −1.957 (0.975) | NS |
| 3 years | −1.864 (1.221) | <0.05 |

BMD, bone mineral density; NS, not significant

Pubertal status, according to Tanner's stages [17], was also evaluated. At the beginning of the study all patients were in Tanner's stage 1, while after 3 years of follow up, all of them showed beginning or advanced (Tanner's stage 2 or 3) puberty.

Associations between parameters were determined by Pearson correlation coefficients and linear regression analyses. Average values were compared with Student's $t$-tests and the $F$ test.

## Results

At baseline, the mean lumbar Z score value was −2.048 SD ± 0.75; the Z score was less than −2 SD in 13 children (59% of the subjects), within −1 and −2 SD in 6 (27.3%), and higher than −1 SD range in only 3 children (13.7%). The mean Z score values increased to −1.957 ± 0.975 at 1 year (not significantly different from baseline) and to −1.864 ± 1.221 at the 3-year follow up ($P < 0.05$ vs baseline; Table 2).

Mean BMD values observed at the lumbar level and at different femoral subregions are shown in Table 3: statistically significant BMD increases from baseline were observed after 1 and 3 years' follow up at all examined sites.

Table 4 shows the mean BMD rate of change/year (expressed both in absolute and percentage values) at the lumbar level and at different femoral subregions observed between the baseline and the 3-year evaluation. A significant increase ($P < 0.0001$) was observed both at the lumbar level (+8.466%/year) and at the femoral subregion level (range, +3.46%/year at the neck to + 5.83%/year at the intertrochanteric subregion) after 3-year follow up.

Regarding the two different iron-chelating treatments, no significant difference in BMD rate of change/year between the two groups of patients was found (Table 5). Moreover, the same trend in bone turnover markers was observed in desferrioxamine- and L1-

**Table 3.** Mean BMD values at lumbar level and at different femoral subregions observed at baseline and after 1 and 3 years' follow up

| | Baseline | 1 year | 3 years | *P* baseline vs 1 year | *P* baseline vs 3 years |
|---|---|---|---|---|---|
| Lumbar L1–L4 | 0.563 ± 0.042 | 0.626 ± 0.067 | 0.710 ± 0.118 | <0.0001 | <0.0001 |
| Femoral neck | 0.646 ± 0.051 | 0.679 ± 0.064 | 0.715 ± 0.093 | <0.005 | <0.0001 |
| Total | 0.644 ± 0.055 | 0.683 ± 0.067 | 0.730 ± 0.097 | <0.0001 | <0.0001 |
| Trochanter | 0.526 ± 0.056 | 0.574 ± 0.052 | 0.592 ± 0.074 | <0.0001 | <0.0005 |
| Intertroch. reg.[a] | 0.687 ± 0.073 | 0.736 ± 0.088 | 0.810 ± 0.129 | <0.0001 | <0.0001 |

[a] Intertrochanteric subregion

**Table 4.** Mean BMD rate of change/year (expressed both in absolute and percentage values) at lumbar level and at different femoral subregions observed between baseline and 3-year evaluation

| | Rate of change/year | Rate of change/year % |
|---|---|---|
| Lumbar L1–L4 | 0.049 | 8.466 |
| Femoral neck | 0.023 | 3.461 |
| Total | 0.028 | 4.388 |
| Trochanter | 0.013 | 4.263 |
| Intertroch. reg. | 0.041 | 5.831 |

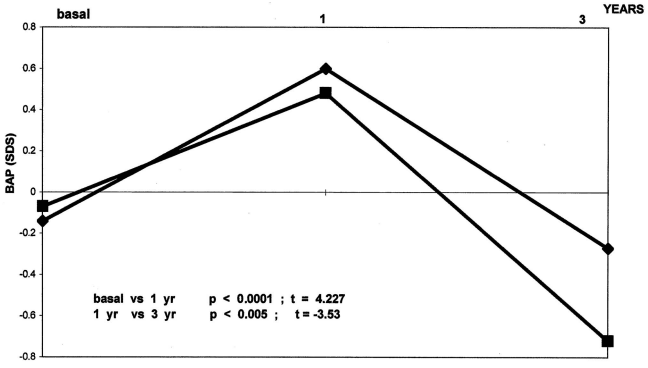


**Fig. 1.** Bone alkaline phosphatase (BAP). ◆, desferrioxamine (DFX); ■, deferiprone (L1)

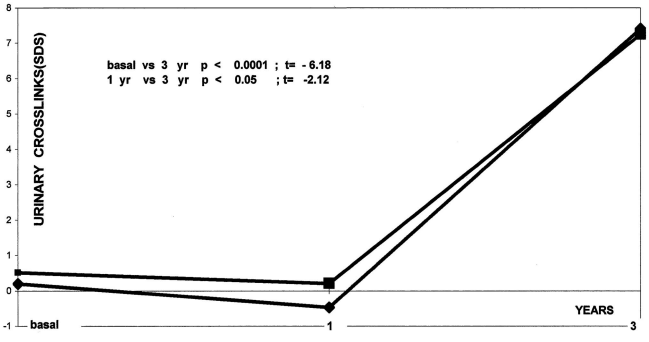


**Fig. 2.** Urinary crosslinks. ◆, DFX; ■, L1

chelated patients. Serum calcium, magnesium, and phosphate levels were within the normal range in all subjects both at baseline and after follow up; BAP values were at the lower limit at baseline, were slightly enhanced after 1 year of follow up, and were reduced after 3 years (these changes were not statistically significant) (Fig. 1); urinary crosslinks values were at the upper limit at baseline, remained unchanged after 1 year, and were significantly increased ($P < 0.05$) after 3 years (Fig. 2).

## Discussion

Our data confirm that, in prepubertal thalassemic children, lumbar and hip BMD levels are markedly lower than values reported for healthy age-matched children [18–20]. The observed increase in bone turnover can probably be attributed to the approach of puberty. It is well known that bone turnover accelerates remarkably during the pubertal period, leading to an important increase in BMD, which is due to a positive bone balance, characterized by high osteoblastic activity.

We observed a significant increase in urinary crosslinks values after 3 years, while bone alkaline phosphatase (BAP) did not change significantly from baseline. Urinary crosslinks and BAP are markers of osteoclastic and osteoblastic activity, respectively; the significant change in urinary crosslinks could be due to the increase of bone turnover induced by the approach of puberty.

We speculate that the small number of subjects in the study, together with the wide variation of the SDs from average, hindered us in proving statistically significant differences; however, the range of SD and BMD values suggests that puberty exerts a remarkable role in bone mass peak achievement not only in healthy children but also in our thalassemic subjects.

According to our results, it is difficult to state which are the most important pathogenetic factors (delayed puberty and hypogonadism, desferrioxamine toxicity, iron overload toxicity, remaining hematopoietic activity with bone marrow enlargement and damage of bone

**Table 5.** Mean baseline BMD and BMD rate of change/year in the group treated with desferrioxamine and in the group treated with deferiprone (L1); all *P* values not significant)

| | Desferrioxamine (SD) | L1 (SD) |
|---|---|---|
| Baseline Lumbar BMD | 0.544 (0.0017) | 0.579 (0.0013) |
| 3-year lumbar BMD | 0.662 (0.0133) | 0.751 (0.0118) |
| Lumbar BMD rate of change/year | 0.039 (0.0007) | 0.057 (0.0008) |
| Lumbar BMD rate of change/year % | 7.075 (20.8) | 9.626 (19.4) |
| Baseline femoral neck BMD | 0.641 (0.001) | 0.651 (0.0039) |
| 3-year femoral neck BMD | 0.699 (0.006) | 0.729 (0.011) |
| Femoral neck BMD rate of change/year | 0.019 (0.0004) | 0.026 (0.0006) |
| Femoral neck BMD rate of change/year % | 2.95 (8.76) | 3.88 (13.5) |

tissue precursor cells, delayed skeletal development due to chronic illness) in osteoporosis in our patients.

We had a rationale for searching for possible differences between the two iron-chelation treatments. First, the reduction in BMD in thalassemia has often been attributed to a toxic effect of desferrioxamine [14,4]. Secondly, the two iron chelators have important differences, in terms of their chemical nature, molecular weight, iron binding (desferrioxamine: Fe is 1:1, whereas deferiprone: Fe is 3:1), and iron excretion patterns. So we planned a longterm prospective comparison of bone mass and metabolism markers between different chelators. Nevertheless, because no significant difference was found between our patients treated with desferrioxamine and those treated with L1 either at baseline or after follow up, the effects of these two treatments on BMD and bone metabolism would seem to be similar. The significant BMD increases observed with both treatments at the lumbar and femoral levels suggest that iron-chelating treatment probably does not exert a significant negative effect on bone mass, and that the low bone mass increases significantly at different skeletal sites, regardless of pharmacological treatment.

Significant BMD increases ($P < 0.0001$) were observed at the lumbar (both in terms of absolute values and in terms of Z scores versus healthy age-matched subjects) and at the femoral level after 3 years of follow up, when all patients showed a beginning or advanced pubertal stage, indicating that, at least at the beginning of puberty, the rate of BMD increase is higher in thalassemic subjects than in healthy age-matched children (according to the reference normative BMD values for children given by our densitometer and some cross-sectional studies [19,21–23]). We did not examine a control age-matched children group during treatment for ethical reasons, because we were concerned about X-ray exposure in normal children; thus, our results could be biased because we did not use age- and pubertal-stage-matched longitudinal data.

The finding that the rate of BMD increase is higher in thalassemic subjects can be explained by the reduction of the negative effects of delayed puberty and hypogonadism on BMD; low BMD in our thalassemic patients is at least partially due to delayed puberty and/or hypogonadism; therefore, the beginning of puberty can contribute to attenuating or reversing the loss of bone mass, leading to an increased BMD.

In any case, a longer follow-up period is required in order to evaluate whether this higher rate of BMD increase characterizes the whole pubertal development period, or only its beginning.

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