Bone mineral status in prepubertal children with constitutional delay of growth and puberty

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Abstract

Objective: We wished to clarify whether the osteopenia reported in adult men with a history of constitutional delay of growth and puberty (CDGP) could be due to the delayed puberty or an independent predisposition to osteoporosis in this condition.

Design: Short prepubertal children with CDGP and children with familial short stature (FSS) were matched for height and other auxological variables. The FSS children served as a control group.

Methods: We measured spinal (L1–L4) bone mineral content (BMC) and bone mineral density (BMD) by dual energy X-ray absorptiometry (Hologic QDR 1000/w) in 56 children aged 5–11 years. All children had height below the 10th percentile for chronological age (CA), and bone age (BA) less than 10 years. 29 of them with clinical diagnosis of possible CDGP and 27 of them with FSS. The BMD standard deviation scores (SDS) relative to the values for normal height children were obtained.

Results: The mean (± s.d.) spinal BMD was significantly lower in the children with CDGP than in the FSS group (0.534 ± 0.059 vs 0.623 ± 0.060 g/cm², P < 0.001). Both groups had negative mean lumbar BMD SDS, but in the CDGP group it was significantly lower than in the FSS group as well when the SDS was based on the CA (−1.41 ± 0.61 vs −0.38 ± 0.51, P < 0.001) and when it was related to BA (−0.78 ± 0.64 vs −0.17 ± 0.52, P < 0.01). BMC was significantly lower in the CDGP than in the FSS group, when multiple regression analysis was performed by using scanned bone area, body weight and height, sex and BA as independent variables (P = 0.0005).

Conclusion: The finding of decreased mineralization in prepubertal children with CDGP before the age of puberty suggests that they may have an inherent predisposition to osteopenia.

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Introduction

Osteopenia has been reported in adult men with a history of constitutional delay of growth and puberty (CDGP) (1). This finding suggests that the timing of sexual maturation is an important determinant of adult bone mineral density (BMD). It is possible, however, that CDGP has an inherent predisposition to osteopenia. To clarify this point we measured the bone mineral content (BMC) and BMD in short prepubertal children with clinical diagnosis of possible CDGP and compared the results with a group of children with familial short stature (FSS) matched for age and auxological parameters. To avoid the possibility of size-related artifacts in the assessment of bone mineral data we followed the procedure advocated by Prentice et al. (2).

Materials and methods

Subject selection

We studied 56 children aged 5 to 11 years, recruited from our Growth Clinic (Hospital 12 de Octubre). All children met the following criteria: (a) height below the 10th percentile for chronological age (CA); (b) Tanner (3) pubertal stage 1; (c) normal birth weight and clinical history and physical examination without evidence of anosmia, micropenis, organic disease, vitamin D deficiency, malnutrition, or psychological deprivation; (d) no intake of medication with a known effect on BMD or growth; (e) bone age (BA) at/or below 9 years in girls and 10 in boys; (f) other causes of short stature excluded by normal blood count and erythrocyte sedimentation rate, electrolytes, creatinine, calcium,
inorganic phosphate, alkaline phosphatase, total protein, thyroxine, thyrotropin, antigliadin antibodies, insulin-like growth factor-I (IGF-I), and uroanalysis; and (g) growth velocity (recorded from a minimum period of 6 months) above the 25th percentile (only in six children was it between the 10th and the 25th percentile, and they showed normal growth hormone (GH) response to exercise and propranolol, and to insulin-induced hypoglycaemia). Informed consent was obtained from parents, as prescribed by the local human ethics committee.

The diagnosis of possible CDGP was made on clinical grounds in children whose BA was at least 1.75 years below their CA and who had a family history of CDGP or mid-parental height above the 25th percentile. Those children with a BA similar to the CA (± 1 year) and a family history of short stature with mid-parental height below the 10th percentile were diagnosed as having FSS. The FSS group was used as the control group instead of normal height children in order to be able to match the anthropometric parameters.

Clinical examination

In every child and its parents, height was measured barefoot on a wall-mounted stadiometer (Holtain Ltd, Crymych, Dyfed, UK) to the nearest 0.1 cm. The children’s weight was measured while they were only in their underwear on a balance scale (Seca, Model 220, Hamburg, Germany) to the closest 0.1 kg. Body mass index (BMI) was calculated as weight divided by square height (kg/m²). The height standard deviation score (SDS) and the BMI SDS were obtained from Spanish sex and age matched reference values (4). The Tanner (3) pubertal stage was determined according to breast development in females and genital development in males. The BA was assessed from a standard X-ray of the non-dominant hand and wrist, evaluated independently by two of the authors (MMA, FJC) using Greulich and Pyle Tables (5); fairly congruous results were obtained, and the mean value was determined. The children’s dietary calcium was estimated from a questionnaire, standardized for children’s use, on the average intake of dairy products and other calcium-rich foods (6). Physical activity was estimated from the number of hours per week of recreational physical exercise.

Bone mineral measurements

BMC and BMD were measured by dual X-ray absorptiometry (DXA) which was performed with a QDR 1000/w densitometer (Hologic, Inc., Waltham, MA, USA; software 6.1). In this DXA unit, collimated X-rays are pulsed at 70 and 140 kV through a calibration disk, then through the region of study, and finally measured by a detector located above the subject. The BMD is calculated on the basis of the subject’s attenuation of the beam in comparison with the reference standard.

The entrance radiation dose to the child was 1–5 mrem, which is less than one-tenth of the exposure from a standard chest X-ray (7, 8). Quality control was performed every day during the study by phantom BMD measurements. The coefficient of variation was 0.3% for the phantom images and 1.3% in vivo.

The anterior/posterior lumbar spine (L1–L4) was scanned with the subject in the supine position, and physiological lumbar lordosis was increased by elevation of the knees. All images were processed by the same researcher. The scanning time for the global region of interest ranged between 5 and 8 min, depending on the age of the child.

Absorptiometric data were reported as the quantity of bone mineral within the scanned area, referred to as BMC (g) and as BMD, derived by dividing the BMC by the scanned bone area (SBA, cm²). Results for BMD were expressed as absolute values (g/cm²) or as SDSs relative to the values for normal height children. The BMD SDS was based on either the CA or the BA of the patients unless stated otherwise.

Statistical analysis

Student’s t-test for unpaired data was used to analyze intergroup differences. Multiple linear regression analysis, by using BMC as the dependent variable and SBA, body weight and height as independent variables, was performed in the analysis of bone mineral data (2). The regression analysis was performed after all continuous variables were converted to natural logarithms. Analysis was performed with SAS v 6.12 software (SAS Institute, Inc., Cary, NC, USA). A level of P <0.05 was considered statistically significant. Values are expressed as mean ± S.D.

Results

Clinical characteristics

Clinical data of children with CDGP and FSS are shown in Table 1.

There were no significant (NS) differences between groups in age, height, height SDS, weight, BMI and BMI SDS. We also found similar results in both groups for the estimated dietary calcium intake (1134 ± 498 vs 1025 ± 503 mg/day) and the reported recreational exercise (3.9 ± 1.3 vs 4.1 ± 1.5 h/week). The CDGP group had significantly lower BA, higher BA delay and higher mid-parental height than the FSS group (Table 1).

Bone mineral data

The group of children with CDGP had a significantly lower mean BMC and lower SBA than the FSS group (26.7% for BMC and 12.3% for SBA); absolute values for boys and girls are shown in Table 2.
The mean spinal BMD was significantly lower in the group of children with CDGP than in the FSS group (0.534 ± 0.059 vs 0.623 ± 0.060 g/cm², P < 0.001). Both CDGP and FSS groups had negative mean lumbar BMD SDS, but in the CDGP group it was significantly lower than in the FSS group as well when the SDS was based on the CA (−1.41 ± 0.61 vs −0.38 ± 0.51, P < 0.001) and when it was related to BA (−0.78 ± 0.64 vs 0.17 ± 0.52, P < 0.01). When results were analyzed separately for boys and girls, the lumbar BMD, expressed as absolute values, as SDS based on CA or as SDS based on BA, was significantly lower in the group of children with CDGP than in the FSS group in boys and in girls (Table 2).

**Correction of bone mineral data for bone and body size**

The relationship between BMC and SBA was analyzed by using BMC as the dependent variable and SBA, body weight and height as independent variables in multiple linear regression analysis. In CDGP and FSS groups the regression coefficients of BMC against SBA were 1.63 and 1.23 respectively (P = 0.0001) (Table 3).

Multiple linear regression demonstrated significant differences in BMC between CDGP and FSS groups after adjustment for height, weight, SBA, sex and BA were made. The significant determinants of BMC were the SBA and the diagnosis. For BMC the mean difference between groups was 2.13 ± 0.57 (t = 3.7, P = 0.0005).

**Discussion**

Our findings are in agreement with those of Finkelstein et al. (1). They reported that adult men with a history of delayed puberty have decreased radial and spinal BMD and suggested that this could be related to the pubertal delay. However, pubertal retardation is of no account in our study, in which none of the children included had any clinical sign of puberty. Since BMD in children correlates with auxological variables (9–12), we did not use normal height children as control group, but a group of short children whose height and weight were similar to the height and weight of the children with CDGP. We found that all the absorptiometric parameters were lower in the group of children with CDGP. We are unaware of previous studies of bone mineralization in children with constitutionally delayed growth or of reported diminished bone size in this condition. Our finding of lower SBA in the CDGP group may not be totally explained by differences in body size between both groups; the FSS boys tended to be heavier than CDGP children, but the differences in body weight between both groups were not significant. It is also

<table>
<thead>
<tr>
<th>Number of subjects</th>
<th>CDGP</th>
<th>FSS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC (g)</td>
<td>14.8 ± 3.2</td>
<td>19.9 ± 3.5</td>
<td>&lt;0.001</td>
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<tr>
<td>SBA (cm²)</td>
<td>26.6 ± 3.5</td>
<td>31.1 ± 3.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>L1–L4 BMC (g/cm²)</td>
<td>0.553 ± 0.06</td>
<td>0.643 ± 0.06</td>
<td>&lt;0.001</td>
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<tr>
<td>L1–L4 BMC SDS for CA</td>
<td>−1.24 ± 0.57</td>
<td>−0.43 ± 0.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L1–L4 BMC SDS for BA</td>
<td>−0.57 ± 0.44</td>
<td>−0.10 ± 0.57</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 2 Spinal (L1–L4) BMC, SBA and BMC in children with CDGP and children with FSS (means ± s.d.).
Table 3. Analysis of bone mineral data in 29 children with CDGP and in 27 children with FSS. Multiple linear regression analysis with three size-related variables (SBA, weight, and height) converted to natural logarithms (ln). Model: ln BMC = constant + ln (SBA) + ln (weight) + ln (height).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>S.E</th>
<th>t ratio</th>
<th>P</th>
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<tr>
<td>CDGP</td>
<td>Constant</td>
<td>-0.52</td>
<td>2.47</td>
<td>-0.21</td>
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<td></td>
<td>ln (SBA)</td>
<td>1.63</td>
<td>0.26</td>
<td>6.18</td>
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<td></td>
<td>ln (weight)</td>
<td>0.19</td>
<td>0.20</td>
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<td></td>
<td>ln (height)</td>
<td>-0.58</td>
<td>0.73</td>
<td>-0.79</td>
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<tr>
<td>FSS</td>
<td>Constant</td>
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<td>1.32</td>
<td>-2.12</td>
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<tr>
<td></td>
<td>ln (SBA)</td>
<td>1.23</td>
<td>0.14</td>
<td>8.69</td>
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<tr>
<td></td>
<td>ln (weight)</td>
<td>0.11</td>
<td>0.14</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>ln (height)</td>
<td>0.25</td>
<td>0.36</td>
<td>0.69</td>
</tr>
</tbody>
</table>

possible that some unrecognized abnormality may affect vertebral size in CDGP.

In normal children BMD is positively correlated to BA (9, 11, 12). BA delay is an important feature in CDGP while in FSS there is no BA retardation. Therefore, since the mean BA of the CDGP group was significantly lower than in the FSS group, the finding of lower BMD in the CDGP group could be expected. However, when we compared the BMD SDS of both groups related to skeletal age we still found lower values in the CDGP than in the FSS group.

Assessing bone mineralization by BMD can be misleading because BMD is not a measure of true density, since absorptiometry provides no information about the depth of bone in the scan path. The recognition that BMD does not adequately correct for bone and body size has led to the development of several approaches to the problem. These include the derivation of volumetric indexes of bone density and of bone mineral apparent density (13, 14). We have applied in our study the approach advocated recently by Prentice et al. (2), which has the advantages that it is a simple way to avoid the possibility of size-related artifacts in the analysis of bone mineral data and that it can be applied in all situations. Expression of data as BMD implies that BMC is directly proportional to SBA. We found that, for our groups of study, BMD was not an appropriate adjustment for bone size because the coefficient of the regression BMC on SBA was >1. The analysis with logged variables has the advantage that the regression coefficients provide information in proportional terms about the influence of each factor on BMC (15). In our study it is evident that a 1% change in SBA is associated with a 1.6% change in BMC in the CDGP group and a 1.2% change in BMC in the FSS group. These results highlight the importance of simultaneous assessment of BMC, BMD and bone size in evaluation of bone mineral status in children.

Because in our population the relationship between BMC and SBA was not one of simple direct proportion, part of the differences in BMD between the CDGP and FSS groups could be due to differences in bone size between individuals. Therefore it was necessary to analyze differences in BMC in both groups after adjustment for SBA and other body size variables. We confirmed that children with CDGP have decreased mineralization compared with FSS children. The underlying cause for BA delay in CDGP is not known. We have shown in this study that this condition has also poor bone mineralization. Whether the BA retardation and the diminished mineralization in CDGP have the same cause remains to be seen. We can only speculate about the possible mechanisms for these findings. The influence of GH/IGF-I on bone mineralization is well documented (16–19) as it is for the influence of sex steroids (20, 21). Causes for CDGP are far from clear. Children with this condition are included, in most studies, in idiopathic short stature, and, whereas normality in the GH secretory dynamics has been reported (22), there are studies that report an impaired GH secretion (23, 24), decreased GH binding proteins (25, 26) and mutations of the GH receptor in some children with idiopathic short stature (27). Therefore, an abnormality in the GH/IGF-I axis cannot be excluded as a possible factor for the poor mineral density found in CDGP. Diminished sex steroids from adrenal glands and delay in adrenarche could also play a role in the lower BMD in children with CDGP. Measurement of androgens as well as bone formation and bone resorption markers in these children would help to understand the underlying cause for their poor mineralization.

In summary, the osteopenia found in adult men with a history of delayed puberty (1) could be due to the delayed sexual maturation and/or to an independent predisposition to osteoporosis in this condition. To rule out the role of delayed puberty, we studied prepubertal children before the age of puberty. Our finding of lower mineralization in these children is consistent with the hypothesis that subjects with CDGP may have some unrecognized abnormality that affects bone mineralization. However, these results cannot be considered definite. Long-term follow-up to confirm the CDGP diagnosis and the outcome of bone mineral status in these children is necessary and is in progress.

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References
2 Prentice A, Parson TJ & Cole TJ. Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the


16 Bernard R, Ng KWW, Martin TJ & Waters MJ. Growth hormone (GH) receptors in clonal osteoblast-like cells mediate a mitogenic response to GH. Endocrinology 1991 132 1031–1037.


21 Holmes SJ & Shalet SM. Role of growth hormone and sex steroids in achieving and maintaining normal bone mass. Hormone Research 1996 45 86–93.


27 Bernard R, Ng KWW, Martin TJ & Waters MJ. Growth hormone (GH) receptors in clonal osteoblast-like cells mediate a mitogenic response to GH. Endocrinology 1991 132 1031–1037.


31 Holmes SJ & Shalet SM. Role of growth hormone and sex steroids in achieving and maintaining normal bone mass. Hormone Research 1996 45 86–93.


37 Bernard R, Ng KWW, Martin TJ & Waters MJ. Growth hormone (GH) receptors in clonal osteoblast-like cells mediate a mitogenic response to GH. Endocrinology 1991 132 1031–1037.


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