Bone mineralization in children and adolescents with type 1 diabetes

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Abstract

Objectives: to evaluate the occurrence of osteopenia and the prognostic factors of bone mass in a pediatric group with type 1 diabetes.

Methods: the following parameters were analyzed in a group of 23 patients with type 1 diabetes aged 10.9 ± 2.9 years: bone mineral density, serum C peptide, glycosylated hemoglobin, serum calcium, serum alkaline phosphatase, serum phosphorus and calciuria. Clinical variables included age, weight, height, body mass index, pubertal stage, insulin doses, duration of diabetes and calcium intake. Bone mineral density was evaluated in the lumbar spine and the results were expressed in deviation standard score by age and sex. Calcium intake was calculated based on feeding report, body mass index was calculated using the “Quetelet” formula and pubertal stage was defined according to the Tanner - Whitehouse criteria. Simple linear regression was used to analyze correlations between variables and the Mann - Whitney U test was used to compare groups.

Results: average bone mineral density was normal (- 0.75 ± 1.01 SD). However we verified that 39.1% of the patients had osteopenia. When comparing data of osteopenic patients (n = 9) to non-osteopenic patients (n =1 4), we observed that C peptide of osteopenic group was higher than that of non-osteopenic group (0.56 ± 0.18 versus 0.29 ± 0.20; p < 0.05). Body mass index and C peptide correlated with bone mineral density. Duration of diabetes was inversely correlated with C peptide (p < 0.01) and directly correlated with insulin doses (p < 0.01).

Conclusion: osteopenia occurred in 39.1% of the patients with type 1 diabetes. The presence of osteopenia was related to higher levels of C peptide.


Introduction

It is during childhood and adolescence that the greater part of the organism’s mineral capital is acquired. During these phases the most significant increases in bone mass are observed. The annual bone mineral density increase (bone mass expressed in g/cm²) is high during the first three years of postnatal life and then progressively diminishes until the onset of puberty. During puberty, the annual increase in bone mineral density (BMD) increases, reaching a maximum at Tanner stages III and IV. After the completion of puberty the annual increase in BMD diminishes progressively, continuing at a decreased rate until a point between 21 and 25 years of age, when BMD stabilizes. At this point the individual attains its peak bone mass. Fifty percent of the total increase in bone mineral density is produced between the first few months of life and the onset of pubertal development, 30% during pubertal development and approximately 20% during late adolescence until an age

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between 21 and 25. From 21 - 25 years onwards, BMD remains constant until the 4th or 5th decade of life when a gradual, physiological loss begins, secondary to advancing age, which, in females, is associated with post and perimenopausal loss.\textsuperscript{1,2}

Osteopenia and osteoporosis refer to two pathological states characterized by a loss of bone mass. According to the World Health Organization, osteopenia is characterized by bone mineral density of between -1 and -2.5 standard deviations from the average attained by young adults. Osteoporosis is defined by a BMD level lower than -2.5 standard deviations from the average, with severe osteoporosis being characterized by bone fractures due to fragility. Within pediatric age groups, a reduction in bone mass is defined as osteopenia, which refers to a BMD below -1 standard deviation from the average for age and sex. The term osteoporosis does not apply to this age group since peak bone mass has not yet been attained.

Considering that 80\% of bone mass is acquired during childhood and adolescence,\textsuperscript{1,2} the pediatric period is critical to the acquisition of skeletal mineral content. The progressive increase in bone mass during this period is due to an anabolic bone state, which is the result of a bone and mineral metabolic pattern characteristic of this age. This pattern can be altered during the course of certain conditions, especially chronic ones, provoking a reduction of bone mass while still within the pediatric age group. A number of different chronic diseases\textsuperscript{3-7} have been related to such a situation, one of which is diabetes mellitus. A reduction in bone mass has been described both in carriers of type 1 diabetes mellitus (DM1) and in carriers of diabetes mellitus type 2 (DM2).\textsuperscript{8-11}

The pathophysiologic mechanisms related to bone loss with DM appear to include a reduction in osteoblast activity, alterations to the metabolism of phosphorous and calcium, a reduction in the collagen synthesis or a reduced production of IGF-I and insulin.\textsuperscript{12-16} Some studies suggest that patients with inadequate metabolic control and a long evolution present a greater risk of osteopenia. However, other authors have not confirmed these findings as there is no consensus among variables related to bone loss in diabetic populations.

We performed this study with the objective of ascertaining whether this reduction in bone mass is present among our group of children and adolescents with DM1 and to study the existence of bone mass prognosis factors.

**Patients and methods**

Twenty-three children and adolescents were studied from a total of 32 patients diagnosed with DM1 according to the American Diabetes Association criteria being treated at the Pediatric Endocrinology Clinic of the Universidade Regional de Blumenau. Nine patients refused to take part in the study and one was excluded due to the presence of artifacts in their densitometric examination. Of the 23 patients studied, nine were female (eight pubescent) and 14 were male (four pubescent). After informed consent was obtained and approved by the Ethics Committee, the patients underwent bone mass evaluation; blood tests for serum calcium levels, alkaline phosphatase, phosphorous, C-peptide and glycosylated hemoglobin (GHb); and urine tests for calciuria. The clinical variables studied were age, weight, stature, body mass index (BMI), stage of puberty, daily calcium ingestion, duration of DM1 and daily insulin dosage. A daily calcium ingestion level of 800 mg/day was considered adequate.

Weight and stature were determined with Filizzola\textsuperscript{TM} anthropometric scales and expressed as standard deviation from the average for age and sex (SD) according to the NCHS growth curves. Stage of puberty was defined according to the Tanner-Whitehouse criteria. BMI was calculated using the “Quetelet” formula: weight (kg) / height\textsuperscript{2} (meters) and expressed in SD from the NCHS curves. Daily insulin doses were divided by body weight and expressed in IU/kg/day, with the total daily dose being taken as the sum of NPH insulin and normal insulin taken daily. In those patients whose regular insulin doses varied depending on capillary glycemia and/or glycosuria, the dosage was calculated based on the number of insulin units used with the greatest frequency. Daily calcium ingestion was evaluated by means of 24 hour dietary recall.

The measurement of serum calcium and phosphorous was performed using the ion selective electrode technique, calcuiuria by the colorometric technique, alkaline phosphatase by enzyme kinetics, C-peptide by chemoilluminescence and GHb by microparticle Elisa. Reference values for these techniques are given in Table 1. Calciuria was divided by body weight and expressed in mg/kg/day.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium</td>
<td>8.5 - 11.0</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Serum phosphorus</td>
<td>2.0 - 6.1</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>56 - 230</td>
<td>U/L</td>
</tr>
<tr>
<td>Glycosylated hemoglobin</td>
<td>4.8 - 7.8</td>
<td>%</td>
</tr>
<tr>
<td>C-peptide</td>
<td>0.9 - 4.0</td>
<td>ng/ml</td>
</tr>
</tbody>
</table>

The bone mass of each patient was evaluated using bone dual energy X-ray densitometry with Lunar DPX - L equipment. Bone mass was measured at lumber vertebrae between L\textsubscript{2} - L\textsubscript{4}. Bone mineral density (BMD) expressed in g/cm\textsuperscript{2}, was later converted into a standard deviation from the average for age and sex, employing normality values
provided by the manufacturer. A BMD level of less than -1 SD was considered osteopenia.

Patients were later divided into two groups depending upon whether or not osteopenia was present in order to study possible differences between the variables being observed. The group of patients with osteopenia was made up of nine patients (four pubescent, three female) and the group of patients without osteopenia contained 14 patients (eight pubescent, six female).

Data was stored and analyzed using the statistical package Stat View II™. To describe the population descriptive statistics were used. In the study of relationships between the different variables simple linear regression was used. The groups were compared using the Mann-Whitney U test. Statistical significance was considered to exist for “p” values equal to or less than 0.05.

Results

Clinical characteristics for the 23 patients studied are presented in Table 2. The daily calcium ingestion level of 16 of the patients was found to be lower than that recommended.

Serum levels of calcium, phosphorous and alkaline phosphatase were normal with averages of 9.4 ± 0.9 mg/dl; 4.3 ± 0.9 mg/dl and 197.0 ± 80.8 U/L respectively. Average calciuria was 2.8 ± 3.1 mg/Kg/day, with a median of 2.0 mg/kg/day. One patient in the group without osteopenia presented elevated calciuria (> 4.0 mg/kg/day). Two patients in the group with osteopenia did not have urine samples taken. The average C-peptide was 0.40 ± 0.2 ng/ml. All of the patients presented C-peptide levels lower than reference levels. Average GHb was 10.6 ± 2.9% with serum levels varying between 6.3 and 17.3%. Two patients presented normal GHb levels.

We observed that the mean average for BMD was normal (-0.75 ± 1.0 SD), varying from -2.96 to 1.08 SD. Nevertheless, we found nine patients (39.1%) to present osteopenia. When comparing the data from patients with osteopenia (n = 9) with data from those without osteopenia (n = 14) we observed that C-peptide for the group with osteopenia was greater than for the group without osteopenia (Figure 1). No significant differences were found between the group(s for the remainder of the variables studied (Table 3). Of the clinical and biochemical parameters studied, only BMI and C-peptide correlated with BMD (Figure 2). The duration of diabetes had a negative relationship with C-peptide (R = -0.590; p < 0.01) and a positive one with the daily insulin dosage (IU/kg/day) (R = 0.562; p < 0.01). There was no statistically significant difference in BMD between pubescent and non-pubescent patients.

![Figure 1](attachment://image.jpg)  
**Figure 1** - Comparison of the serum levels of C-peptide (mean SD) in both groups of patients

**Table 2** - Clinical characteristics of the patients (n = 23)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>S.D.*</th>
<th>Gap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>10.9</td>
<td>2.9</td>
<td>5.0 - 16.3</td>
</tr>
<tr>
<td>Duration of diabetes (months)</td>
<td>40.0</td>
<td>34.2</td>
<td>3 - 123</td>
</tr>
<tr>
<td>Insulin dosage (IU/kg/day)</td>
<td>0.92</td>
<td>0.26</td>
<td>0.30 - 1.29</td>
</tr>
<tr>
<td>Calcium daily intake (mg/dia)</td>
<td>584.0</td>
<td>261.0</td>
<td>224 - 1074</td>
</tr>
<tr>
<td>Weight (SD)</td>
<td>-0.47</td>
<td>0.63</td>
<td>-1.29 - 0.70</td>
</tr>
<tr>
<td>Height (SD)</td>
<td>-0.67</td>
<td>0.53</td>
<td>-1.50 - 0.21</td>
</tr>
<tr>
<td>BMI (SD)</td>
<td>0.03</td>
<td>0.86</td>
<td>-1.50 - 2.00</td>
</tr>
</tbody>
</table>

* standard deviation.
Despite a significant correlation between BMD versus BMI and C-peptide, no specific cut-off value was observed for either BMI or C-peptide above which all of the patients with osteopenia were to be found.

### Table 3 - Comparison between both groups of patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-osteopenic (n = 14) (mean SD)</th>
<th>Osteopenic (n = 9) (mean SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>10.9 ± 2.2</td>
<td>10.3 ± 3.8</td>
</tr>
<tr>
<td>Duration of diabetes (months)</td>
<td>50.0 ± 7.1</td>
<td>25.4 ± 21.3</td>
</tr>
<tr>
<td>Insulin dosage (UI/kg/day)</td>
<td>0.94 ± 0.22</td>
<td>0.89 ± 0.31</td>
</tr>
<tr>
<td>Calcium daily intake (mg/day)</td>
<td>564.2 ± 244.7</td>
<td>617.4 ± 298.8</td>
</tr>
<tr>
<td>Height (SD)</td>
<td>-0.75 ± 0.47</td>
<td>-0.87 ± 0.53</td>
</tr>
<tr>
<td>BMI (SD)</td>
<td>0.38 ± 0.92</td>
<td>-0.38 ± 0.69</td>
</tr>
<tr>
<td>C-peptide (ng/ml)</td>
<td>0.29 ± 0.20</td>
<td>0.56 ± 0.18*</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (%)</td>
<td>10.0 ± 3.7</td>
<td>11.4 ± 2.9</td>
</tr>
</tbody>
</table>

* p < 0.05

Bone mass loss has been reported in patients with DM1 within different age groups. This loss has been described both from cortical and trabecular bone tissue. Within the pediatric age group, reports are found of reductions in trabecular bone mass in isolation or associated with cortical bone mass reductions. This study confirms the findings of reduced trabecular bone mass in children and adolescents with DM1. In the group studied, 39% of the patients presented osteopenia of the lumbar vertebrae. In the literature reviewed this figure can vary from 30 to 50%.

Earlier studies have differed with respect to the association between poor metabolic control and the presence of osteopenia. Some studies have demonstrated such an association, while others have not. We did not find any correlation between BMD and serum levels of GHb. It is possible that the size of the sample studied made it difficult to obtain a significant relationship between these variables, or, that in this group this factor is not involved in the genesis of osteopenia.

Bone Mineral Density was not related to the duration of the disease. Studies which evaluated this relationship disagree. It appears, however, that the loss of bone mass related to diabetes occurs during different phases of the disease and may be of early or late onset. The bone mass loss found in patients with recent onset DM1 suggests to us that bone metabolism is compromised by the disease itself. MacNair et al., studying 215 diabetics, of all ages, using insulin demonstrates that bone loss related to the disease occurs during the first five years of its evolution. There are reports of the presence of osteopenia in children who have had DM1 for less than two years. In this study, 90% of the patients with osteopenia had had the disease for less than five years, which demonstrates that, in the group studied, bone loss is an early finding. Recently Gunczler et al. have also demonstrated that bone mass loss is an early finding with DM1.

The exact mechanism responsible for bone loss in diabetes patients is not yet completely explained. Some studies show that during the growth phase, this loss is due to a low bone metabolism rate. Bone metabolism markers were found to be within normal limits for the group.
of patients with osteopenia and also for the group without osteopenia. Some studies suggest that osteopenia in type 1 diabetics is the result of low levels of insulin or of IGF-I. Both of which have an anabolic effect on bone metabolism, the reduction of which would be compatible with the state of decreased bone metabolism described above. The presence of higher C-peptide levels among the group of patients with osteopenia and the inverse relationship between serum C-peptide levels and BMD may suggest that insulinopenia is related to bone loss in these patients. Therefore, the initial phases of the disease, when C-peptide levels are higher, are when insulinopenia, which precedes DM1 diagnosis, is most closely approached. All of which leads us to think that, in the patients studied, this is the factor which determines bone loss. As was mentioned earlier, osteopenia is an early finding among the group of patients studied, which corroborates this hypothesis. MacNair et al., while not correlating C-peptide with BMD, demonstrated a progressive decline in bone mass loss over the years of DM1 evolution. These factors do not rule out the possibility that there will be future bone loss related to poor metabolic control a number of years into the evolution of the disease as is described in literature. It is probable that bone mass losses related to poor metabolic control will occur after many years of evolution, infrequently having an effect within the pediatric age group. Findings of BMD gain with hyperinsulinism and in diabetic patients with terminal nephropathy where insulin breakdown is reduced, reinforces the idea that insulinopenia is implicated in the genesis of osteopenia with type 1 diabetes.

It is probable that bone mass losses due to diabetes are multifactorial processes as has been suggested by Martinez et al. Therefore, a number of different factors may be involved, in conjunction or in isolation, in the appearance of osteopenia in these patients, and may vary depending upon populational characteristics. The low levels of calcium ingestion found among the patients studied may be an additional factor in the genesis of osteopenia since it is well established in extant literature that adequate calcium ingestion is essential to adequate mineral acquisition. Low observed levels of calcium call attention to a nutritional deficiency the correction of which would eliminate one further risk factor for osteopenia in the group studied.

In conclusion, the results of this study show that osteopenia present in children and adolescents with DM1, occurs during the first years of the disease’s evolution and is related to high serum C-peptide levels. The presence of osteopenia in this age group may compromise the peak bone mass attained by these children; as such long term follow-up of them would allow us to know if they will achieve adequate peak bone mass during adulthood. The correction of daily calcium ingestion levels could eliminate an additional risk factor for bone mass loss within this group. Taking into consideration the small size of the sample studied, future studied involving greater numbers of patients would be useful for confirming these findings.

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References

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