Effect of a gluten-free diet on bone mineral density in children with celiac disease

Effet d’un régime sans gluten sur la densité osseuse chez l’enfant atteint de maladie cœliaque

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Available online 6 February 2009

Summary

Aim. — The aim of the study was to assess the evolution of bone mineral density (BMD) in children with celiac disease and to evaluate the effect of a gluten-free diet (GFD).

Methods. — Altogether, 44 children (31 girls and 13 boys) were followed-up. BMD was measured by dual-energy X-ray absorptiometry of the lumbar spine (Hologic QDR 4500). Results are expressed as absolute values for BMD, and as Z scores for chronological age (BMD/CA) and bone age (BMD/BA). Patients were divided into two groups according to whether they followed a diet without (n = 34) or with (n = 10) gluten for at least 1 year. All patients were clinically free of symptoms at the end of the follow-up.

Results. — At inclusion, 26 patients (59%) were delayed in bone age, 17 children (38%) had a BMD/CA ≤ 1 S.D. and six (13.6%) had a BMD/CA ≤ 2 S.D., whereas nine children (20%) had a BMD/BA ≤ 1 S.D. and three (6.8%) had a BMD/BA ≤ 2 S.D. During the follow-up, the BMD increase was greater in the GFD group, as determined by the BMD/CA/year (+0.05 ± 0.3 vs −0.34 ± 0.4 S.D.; P < 0.01) and BMD/BA/year (−0.02 ± 0.4 vs −0.4 ± 0.6 S.D.; P < 0.05). The gain in BMD/BA was smaller in the GFD group because of their need to catch up in bone maturation.

Conclusion. — Celiac children not following a GFD show delays in both bone maturation and mineralization. This prospective study confirms the importance of maintaining a GFD in children with celiac disease until the end of skeletal mineralization even in asymptomatic patients following a non-restricted diet.

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Introduction

Celiac disease (CD) is defined as an intolerance to gluten with total villous atrophy in the small intestinal mucosa. The extent of tissue damage can range from a short to a long segment of small intestine. It is one of the primary causes of malabsorption in children in Europe [1]. Patients without malabsorption symptoms currently represent the majority of all diagnosed patients, which is why CD is often underdiagnosed, even in childhood. Untreated, CD is associated with malabsorption of calcium and vitamin D, leading progressively to a decrease in bone mineral density (BMD) that, in turn, can lead to osteopenia or even osteoporosis [2,3]. Also, a lack of bone mineralization is frequently associated with a delay in bone maturation at the time of diagnosis [4], which means that BMD can be expressed as Z scores for both chronological age (BMD/CA) and bone age (BMD/BA).

However, although it is well accepted that a gluten-free diet (GFD) can resolve histological abnormalities and clinical symptoms [5], its effects on bone mineralization are a subject of continuing debate. Several authors have shown that a strict GFD allows BMD to return to normal — albeit after a time period that is yet to be defined [6–9] — whereas others state that a strict diet alone is not enough to bring about complete normalization of bone density [1,10,11].

The objectives of our study were to prospectively monitor BMD in children with CD, and to analyze the influence of a GFD on both bone mineralization and maturation.

Patients

This prospective study, conducted between January 2000 and December 2006 at the University Hospital of Rennes, included all children with CD whatever their clinical presentation at diagnosis or disease duration. The diagnosis of CD was established according to criteria of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [12]. The criteria for inclusion were:

- CD diagnosed before the age of 18;
- the presence of total villous atrophy of the duodenal mucosa on biopsy;
- the presence of antigliadin IgA antibodies, antiendomysium IgA antibodies or antitransglutaminase IgA antibodies without IgA deficiency;
- clinical remission after elimination of gluten from the diet.

The exclusion criteria were:

- suspected CD, but not confirmed by histopathology;
- the coexistence of other chronic diseases (insulin-dependent diabetes, inflammatory bowel disease, rheumatic disorders);
- long-term treatment with corticosteroids that could alter bone mineralization.

Cases of CD diagnosed after familial screening were also excluded.

Methods

Each child underwent two assessments with a minimum interval of one year in between. Clinical examination included weight and height expressed as units of standard deviation (S.D.) for age. Compliance with GFD was monitored by medical and dietitian interviews, clinical evaluation, and measurements of antigliadin and antitransglutaminase antibodies. GFD was considered to be not followed in cases of free regimens, lapses admitted in...
Bone mineral density and celiac disease

Table 1 Main symptoms according to age at diagnosis.

<table>
<thead>
<tr>
<th>Symptoms at diagnosis</th>
<th>E group (n = 27)</th>
<th>L group (n = 17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth retardation</td>
<td>27 (100%)</td>
<td>13 (76%)</td>
<td>NS</td>
</tr>
<tr>
<td>Height (≤ 2 S.D.)</td>
<td>2 (7%)</td>
<td>5 (29%)</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (≤ 2 S.D.)</td>
<td>15 (55.5%)</td>
<td>4 (23.5%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Vomiting, anorexia</td>
<td>21 (78%)</td>
<td>5 (29%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>16 (59%)</td>
<td>9 (53%)</td>
<td>NS</td>
</tr>
<tr>
<td>Behavioural abnormalities</td>
<td>12 (44%)</td>
<td>4 (23.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>11 (65%)</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

E group: before 24 months; L group = after 24 months; NS = not significant.

Medical or dietitian interviews, or positive antibodies after GFD of more than one year.

A bone-age X-ray (of the left wrist and hand) was taken on the same day as bone mineral densitometry, and expressed as a Z score for chronological age and bone age. Bone age was analyzed using WW Greulich and SI Pyle's Radiographic Atlas of Skeletal Development of the Hand and Wrist by a pediatric endocrinologist (M de K). Bone age was considered delayed when it was less than the chronological age. BMD was measured by dual-energy X-ray absorptometry at the lumbar spine (L1—L4) in the dorsal decubitus position (Hologic QDR 4500 Elite) and always interpreted by the same operator. The results were expressed in absolute values as g/cm² (BMD), and as Z scores for chronological age (BMD/CA) and bone age (BMD/BA) in units of S.D.

Blood tests for total serum calcium, ionized calcium, phosphorus, alkaline phosphatase, 25-hydroxy vitamin D and intact parathyroid hormone levels were performed at each hospital visit. Serum calcium was measured by atomic absorption spectrophotometry. Serum phosphate and serum alkaline phosphatase levels were measured using standard methods, and 25-hydroxy vitamin D and intact parathyroid hormone levels were determined by radioimmunoassay.

Regarding age at CD diagnosis, the children were divided into two groups:

- the E group (early) was diagnosed before age 24 months;
- the L group (late) was diagnosed after age 24 months.

After assessing the follow-up of GFD between CD diagnosis and the first visit, we allocated the study population into two groups along with their first BMD assessment:

- the A1 group strictly followed the GFD between the diagnosis and first evaluation;
- the B1 group either lapsed or failed to comply with the GFD or showed the presence of specific antibodies, between the diagnosis and first evaluation.

Then, after prospectively observing the GFD follow-up between the two evaluations by bone densitometry, we created the following two groups:

- the A2 group strictly followed the GFD between evaluation 1 and evaluation 2;
- the B2 group either lapsed or failed to comply with the GFD, or showed the presence of positive antibodies, between the two evaluations.

Statistical analyses were carried out using STATISTICA 7.0 software. Normality was tested using Kolmogorov—Smirnov tests. Data (weight, height, bone age and bone density) were compared between groups using either Student's unpaired t test (parametric data) or the Mann—Whitney U test (non-parametric data). Pearson (or Spearman for non-parametric data) rank-order correlation coefficients were used to detect correlations between variables, and P < 0.05 was considered to be statistically significant. Results are reported as means ± S.D., and as minimum and maximum values (m and M, respectively) ± S.D., unless otherwise indicated.

Results

Forty-four children were selected between January 2000 and December 2006 to participate in the present study.

Diagnostic conditions

Mean age at diagnosis for CD was 3.3 ± 3.5 S.D. (0.6; 13.9 years), including 13 boys (4.1 ± 5 years; 0.6—13.9) and 31 girls (3.3 ± 3 years; 0.6—12.3); 27 children were diagnosed before the age of 24 months (E group). In this group, mean weight was −1.8 ± 0.7 S.D. (−3; −0.5) and mean height was −0.6 ± 1.1 S.D. (−3; +2). Seventeen children were diagnosed after the age of 24 months (L group) with a mean weight of −1 ± 1.4 S.D. (−4; 1.5) and mean height of +0.9 ± 1.3 S.D. (−3; +1.5). The main symptoms of these patients are presented in Table 1.

Overall results of the first assessment

Chronological age at the first check-up was 8.2 ± 4.5 years (range 2.3—17.7 years). Mean delay between diagnosis and the first check-up was 4.9 ± 4.3 years (range 0.1—15.5 years; the delay was above 1 year in 36 cases). All patients but three (each following the diet for only one month) were symptom-free. Total serum calcium, ionized calcium, phosphorus, alkaline phosphatase and parathormone levels were normal in all patients.

Results according to age at diagnosis are presented in Table 2. Mean bone age was 7.7 ± 4.5 years (1.6; 16.5 years).

After this time, 26 children (59%) had delayed bone age (52% in E group and 70% in L group; not significant [NS]). Mean BMD of the lumbar spine was 0.591 ± 0.172 g/cm² (0.399; 0.999), mean BMD/CA was −1 ± 0.8 S.D. (−2.5 ± 1.1) and
Mean BMD/BA was $-0.6 \pm 0.9$ S.D. ($-2.3$; $+1.2$). Seventeen children (38%) had a BMD/CA $\leq 1$ S.D. while nine (20%) had a BMD/BA $\leq 1$ S.D. Six children (13.6%) had a BMD/CA $\leq 2$ S.D. and only three (6.8%) had a BMD/BA $\leq 2$ S.D. The L group had a significantly ($P<0.01$) lower BMD/CA than the E group, whereas there was no significant difference between the two groups in BMD/BA.

Results according to the follow-up of the gluten-free diet between diagnosis and the first check-up are presented in Table 3. A total of 33 children strictly followed the GFD (A1 group) while 11 did not (B1 group). Children with poor compliance with the GFD (B1 group) were older than those in the A1 group ($P<0.05$). The delay from CD diagnosis was not statistically different (4.1 $\pm$ 4.8 years vs 4.3 $\pm$ 3.7 years, respectively), and there was no significant difference between the two groups in either height or weight. In all, 22 children (66%) had a delayed bone age in the A1 group and five children (45%) in the B1 group.

The BMD expressed as a Z score for chronological age (BMD/CA) was significantly lower in the A1 group ($P<0.05$), as was the percentage gain in BMD ($P<0.02$). Both the BMD/CA and BMD/BA gains per year in the A2 group were also significantly greater than in the B2 group.

Total serum calcium, ionized calcium, phosphorus, alkaline phosphatase and parathormone levels reverted to normal in all patients.

### Discussion

It is well accepted that untreated CD leads to delays in growth as well as in bone maturation, and both are often present at the time of diagnosis [4,13]. Intestinal malabsorption in particular affects calcium metabolism, leading to a negative calcium balance [14] that can result in osteopenia or even osteoporosis [15]. The decreased absorption of calcium induces secondary hyperparathyroidism, which is also aggravated by a low-calcium diet and can lead to bone demineralization [16–18]. However, serum calcium levels do not usually fall because of diminished renal excretion of calcium. The study by Molteni et al demonstrated this phenomenon by measuring strontium absorption at the time of CD diagnosis, which was clearly lower in celiac patients compared with a control population. In addition, there was an associated diminution of urine calcium levels [14].

### Longitudinal follow-up

A total of 34 children strictly followed the GFD during the longitudinal study (A2 group) while 10 children did not (B2 group). One patient in group B1 became compliant with the GFD during the study and was moved into group A2, while GFD status for all the others did not change. Follow-up duration and the interval between the two assessments did not differ between the two groups ($1.9 \pm 0.9$ vs $1.4 \pm 0.4$ years).

All patients were clinically symptom-free at the time of the second evaluation (Table 4). The average annual gain in BMD was significantly higher in the A2 group than in the B2 group ($+0.033 \pm 0.025$ vs $+0.003 \pm 0.047$ g/cm² per year, respectively; $P<0.05$), as was the percentage gain in BMD per year in the A2 vs B2 group ($P<0.02$). Both the BMD/CA and BMD/BA gains per year in the A2 group were also significantly greater than in the B2 group.

Table 2 Results of first evaluation of bone age and BMD, according to age at diagnosis.

<table>
<thead>
<tr>
<th>First assessment</th>
<th>E group ($n=27$)</th>
<th>L group ($n=17$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>10 $\pm$ 4.2</td>
<td>7.5 $\pm$ 4.4</td>
</tr>
<tr>
<td>Delay from diagnosis (years)</td>
<td>1.7 $\pm$ 2.3</td>
<td>1 $\pm$ 1.42</td>
</tr>
<tr>
<td>Delay of bone age n (%)</td>
<td>14 (52%)</td>
<td>12 (70%)</td>
</tr>
<tr>
<td>Median (years)</td>
<td>$-0.3 \pm 1.3$</td>
<td>$-1 \pm 1.1$**</td>
</tr>
<tr>
<td>BMD/CA (S.D. units)</td>
<td>$-0.7 \pm 0.8$</td>
<td>$-1.4 \pm 0.7$*</td>
</tr>
<tr>
<td>BMD/BA (S.D. units)</td>
<td>$0.5 \pm 0.9$</td>
<td>$-0.8 \pm 0.9$</td>
</tr>
</tbody>
</table>

Values are presented as means $\pm$ S.D. (standard deviation).

Table 3 Mean values for age, weight, height and bone mineral density (BMD) according to compliance with gluten-free diet (GFD) between diagnosis and first bone examination.

<table>
<thead>
<tr>
<th>Group</th>
<th>A1 ($n=33$)</th>
<th>B1 ($n=11$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.4 $\pm$ 3.8</td>
<td>11 $\pm$ 5.1</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>Weight (S.D. units)</td>
<td>0.2 $\pm$ 1.3</td>
<td>0.5 $\pm$ 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Delay from diagnosis (years)</td>
<td>4.3 $\pm$ 3.7</td>
<td>4.1 $\pm$ 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Height (S.D. units)</td>
<td>0.3 $\pm$ 1.1</td>
<td>0.5 $\pm$ 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Bone age (years)</td>
<td>6.7 $\pm$ 3.8</td>
<td>10.7 $\pm$ 5.3</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.545 $\pm$ 0.140</td>
<td>0.725 $\pm$ 0.196</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>BMD/CA (S.D. units)</td>
<td>$-1.1 \pm 0.8$</td>
<td>$-0.5 \pm 0.8$</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>BMD/BA (S.D. units)</td>
<td>$-0.7 \pm 0.9$</td>
<td>$-0.3 \pm 0.8$</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as means $\pm$ S.D. (standard deviation); NS = not significant. BMD = bone mineral density; BMD/CA = BMD expressed as Z score for chronological age; BMD/BA = BMD expressed as Z score for bone age; A1 = good compliance with GFD; B1 = poor compliance with GFD.
Chronic inflammation may also play a role in the pathogenesis of osteopenia as a result of increased levels of cytokine production that stimulate osteoclastic activity. The intestinal mucosa of CD patients is the site of production of proinflammatory cytokines ( interleukin [IL]-1β, IL-6 and tumor necrosis factor-α). Moreover, Fornari et al. found a positive correlation between the incremental gain in BMD and the decrease in IL-6 levels after 37 months of GFD [19]. These data suggest that cytokines are involved in the development of osteopenia, and they have also been proposed as risk factors for osteopenia in inflammatory bowel disorders such as Crohn’s disease [20].

Although numerous studies of the outcome of BMD have been carried out in adults with CD and the results of GFD have been controversial, the findings appear to be linked to the patient’s age at the time of diagnosis [21]. Bone mass acquisition mainly takes place during the first two years of life and in adolescence before the end of puberty. Scotta et al. observed that the BMD of children and adolescents with CD was lower when the GFD was started after the age of two years [22]. According to our data, the BMD/CA was higher in children diagnosed before age two years. This confirms that the peak bone mass acquisition is of great importance in childhood [23] and underscores the need for early diagnosis. The specific age of peak bone mass acquisition remains debatable, but BMD appears to increase in adolescence in parallel with bone maturation and puberty [24,25].

Considering that a delay in bone maturation is frequently found at the time of CD diagnosis and persists thereafter, it would appear to be important to evaluate BMD as a Z score corrected for bone age rather than for chronological age. However, studies on CD do not take this factor into account when evaluating BMD [1,10,23,26,27]. The Herzog et al. study of Crohn's disease also used BMD expressed as a Z score for bone age [28]. In fact, bone-age delay was commonly seen in our study and suggests overestimation of osteopenia if this delay in bone age is not accounted for. The delay is even greater in late diagnoses and the BMD/BA is not as consistently low as the BMD/CA. Paradoxically, the delay in bone maturation may have been more important at the time of the first examination in the A1 group than in the other groups. We believe this was because the GFD did not have enough time to compensate for the delayed bone maturation as the children were younger. At the time of the first examination, the mean BMD/CA was lower in the B1 group, but correcting for bone age reduced this difference, given the greater delay in bone maturation in these children. Thus, correcting for the delay in bone age clearly attenuates the differences in BMD/BA.

The wide variations seen in terms of diagnostic conditions, age, diagnosis delay and GFD duration was a major disadvantage of our study — as with most of the previously published data — and probably explains the heterogeneity among the earlier results. Thus, the need for a prospective study seemed necessary to answer the question of GFD benefit to bone mineralization. During this longitudinal survey, despite the small sample size of the B2 group (patients not following the GFD and no possibility of randomization of GFD), we were able to find a clear benefit with the GFD. We observed a gain in BMD in absolute values and in percentage increments per year, as well as in BMD/CA and BMD/BA. The annual increases in BMD absolute values were not higher than the expected physiological gain in healthy children of the same age (0.02–0.05 g/cm² per year) [29]. However, Tau et al. reported a larger increase in BMD in CD children after 1 year of GFD compared with that expected in non-CD subjects [6].

GFD appears to favor bone maturation as well as increase BMD/BA. Gemme et al. reported that, even after 3 years of GFD started early, the lag in bone age persisted [4]. On the other hand, Barera et al. showed that, after only 1 year of GFD, the BMD of prepubertal children with CD did not differ from that of non-CD children of the same age and gender [15]. This may be because, although the peak bone mass, particularly in girls, is acquired in the 2 years preceding puberty, in a child with CD diagnosed during or after puberty, it takes more time to recover the normal BMD even with the GFD [27,30]. The GFD is, thus, an indispensable element for catching up with bone maturation and BMD, not to mention its other beneficial effects on growth as described elsewhere [15].

In our study, all of the children had normal serum calcium and vitamin D levels, despite highly variable programs of vitamin and calcium supplementation. Other studies have shown that patients with hypocalcemia at the time of CD diagnosis have chronic malabsorption [6]. Vitamin and calcium supplementation is apparently indispensable when the celiac child is not following a GFD, but it is not enough on its own to either improve BMD or compensate for the deleterious effects of an unrestricted diet [2,14].

### Table 4 Annual gain in BMD in absolute values, percentages, BMD/CA and BMD/BA, according to compliance with gluten-free diet (GFD) between the two evaluations.

<table>
<thead>
<tr>
<th>Group</th>
<th>A2 (n = 34)</th>
<th>B2 (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (per year)</td>
<td>+0.12 ± 0.42</td>
<td>+0.10 ± 0.65</td>
<td>NS</td>
</tr>
<tr>
<td>Height gain (per year)</td>
<td>+0.07 ± 0.36</td>
<td>−0.08 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>Bone age gain (years/year)</td>
<td>+0.15 ± 0.4</td>
<td>−0.07 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>BMD gain (g/cm²/year)</td>
<td>+0.033 ± 0.025</td>
<td>+0.003 ± 0.047</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMD gain (%/year)</td>
<td>+3.3 ± 2.5</td>
<td>+0.6 ± 4.5</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>BMD/CA gain (per year)</td>
<td>+0.05 ± 0.35</td>
<td>−0.34 ± 0.47</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMD/BA gain (per year)</td>
<td>−0.02 ± 0.47</td>
<td>−0.4 ± 0.6</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D. (standard deviation); NS = not significant. BMD = bone mineral density; BMD/CA = BMD expressed as Z score for chronological age; BMD/BA = BMD expressed as Z score for bone age; A2 = good compliance with GFD; B2 = poor compliance with GFD.
Conclusion

CD has an overall retarding effect on growth, bone maturation and BMD, and the delay is accentuated when the diagnosis is late. Therefore, it is important to have an early diagnosis and to begin a GFD as soon as possible to prevent the development of osteopenia. Our study confirms the need for a GFD to be followed up to the end of the growth period even in asymptomatic patients, and the usefulness of regular bone-age evaluation in children with CD. Monitoring bone maturation allows evaluation of BMD values as a Z score for bone age, the best estimation of bone status in children. Longitudinal follow-up of BMD provides data on the patient’s risk of osteoporosis, its prevention and treatment. Thus, an insufficient increase in BMD, especially BMD expressed as a Z score for bone age, may be an important factor in discussions of whether or not to restart a GFD that was either interrupted or irregular despite the absence of clinical symptoms or other nutritional deficiencies.

References