Longitudinal changes in bone metabolism and bone mineral content in children with celiac disease during consumption of a gluten-free diet1–3

Graziano Barera, Sabrina Beccio, Maria Carla Proverbio, and Stefano Mora

ABSTRACT
Background: A gluten-free diet (GFD) rapidly corrects the bone mineral deficit of children with untreated celiac disease. The mechanisms underlying such changes are still poorly understood.
Objective: In a longitudinal study, we monitored changes in bone metabolism during consumption of a GFD.
Design: We studied 22 white patients with celiac disease (11 girls) aged 10.5 ± 1.0 y at the time of diagnosis. We compared bone metabolism and bone mass values in these patients with those in 428 healthy white children aged 11.3 ± 0.2 y. Bone-specific alkaline phosphatase (a bone formation index) and N-terminal telopeptide of type I collagen (NTx; a bone resorption marker) were measured at the time of diagnosis and after 2, 6, and 12 mo of the GFD. Bone mineral content was measured at the lumbar spine and for the whole skeleton.
Results: The bone mineral content of patients was significantly lower than that of control subjects at the time of diagnosis but not after 1 y of the GFD. Serum bone-specific alkaline phosphatase concentrations of patients were significantly lower than those of control subjects at the time of diagnosis (P = 0.0064) and increased gradually and significantly during the GFD (ANOVA, F = 4.71; P = 0.024). Conversely, patients with untreated disease had significantly higher urinary concentrations of NTx than did healthy control subjects (P < 0.0001). Urinary concentrations of NTx were not significantly affected by treatment (P = 0.37).
Conclusions: The rate of bone metabolism is altered in children with untreated celiac disease, and these alterations may be the cause of osteopathy. Remarkable changes occur after the initiation of a GFD, and they result in a more balanced equilibrium.

KEY WORDS Bone formation, bone resorption, bone mineral density, bone-specific alkaline phosphatase, celiac disease, children, gluten-free diet, N-terminal telopeptide of type I collagen

INTRODUCTION

Celiac disease is a permanent intolerance to gluten. In susceptible individuals, dietary exposure to the antigen present in wheat, rye, and barley promotes the loss of villous cells in the proximal intestine, which results in impaired absorption of nutrients (1). Celiac disease can be not only overt, with the classic features of chronic diarrhea and weight loss, but also subclinical, with isolated nutrient deficiencies but no gastrointestinal symptoms. Frequent nonmalignant clinical complications include iron deficiency anemia and osteoporosis. Early recognition of the disease is important, because complications can be prevented by adherence to a gluten-free diet (GFD).

Markedly reduced bone mineral content (BMC) and bone mineral density have been repeatedly found in children and adolescents with untreated celiac disease, regardless of clinical presentation (2–4). The axial, peripheral, and whole skeleton seem to be equally affected. The effect of dietary treatment is quite rapid, and children consuming a GFD show normal BMC or bone mineral density measurements even after only 1 y of treatment (2–10). However, the mechanisms that underlie such changes are still poorly understood.

Growth of the skeleton and increase of bone mass are achieved by the coordinated activity of 2 different bone cells: osteoblasts, which synthesize new bone matrix and promote bone formation, and osteoclasts, which are devoted to bone resorption (11). The coupled activity of these cells persists throughout life and during growth promotes changes in the length and shape of bones (bone modeling) (12). The bone metabolism rate can be precisely assessed by histomorphometry of the iliac crest, which is the gold standard for estimating the status of bone turnover (13). However, bone biopsy is an invasive procedure that is not feasible for routine use in the evaluation of bone metabolism. More readily available to physicians are some biochemical tests performed on blood or urine samples, which mirror the ongoing bone metabolic processes (14). These biochemical markers are based on the measurement of either an enzymatic activity characteristic of the bone-forming or bone-resorbing cells or bone matrix components released into the circulation during bone apposition or resorption. The concentration of bone metabolism markers changes markedly during childhood and adolescence. Maximum concentrations are observed in infancy and during the pubertal period, when skeletal growth is more rapid (15–17).

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TABLE 1
Age and anthropometric measurements of patients with celiac disease at the time of diagnosis and during the first year of treatment and of healthy control children

<table>
<thead>
<tr>
<th>Patients (n = 22)</th>
<th>Healthy children (n = 428)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>10.5 ± 1.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>30.9 ± 2.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>135.7 ± 5.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.1 ± 0.4</td>
</tr>
</tbody>
</table>

Only a few studies have monitored the bone metabolism rate in young patients with celiac disease. Bone formation markers have been measured in one short-term longitudinal study (18), whereas rates of bone formation and bone resorption were assessed by measurement of biochemical indexes in children and young adults consuming a long-term GFD (8, 19). The lack of knowledge about the changes in the bone metabolism rate that occur during the first year of treatment prompted us to study longitudinally from the time of diagnosis a group of children with celiac disease.

SUBJECTS AND METHODS

Subjects

Eligible for the study were patients with celiac disease diagnosed according to the recommendations of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (20). Briefly, all patients were identified by clinical symptoms or through screening programs. All showed positive results on tests for antiendomysium antibodies; mucosal alterations consistent with Marsh type II or III lesions in small-bowel biopsy samples, regardless of clinical presentation; and clinical improvement after the initiation of dietary treatment. Twenty-six patients agreed to participate in the study, and 22 (11 girls) completed the 1-y follow-up. The characteristics of the patients are shown in Table 1. The treatment consisted of a GFD. Supplemental iron and folate were provided when indicated. Calcium and vitamin D supplements were not given or recommended.

As a control group, we studied 428 white volunteers (191 girls) aged 11.3 ± 0.2 y who were recruited from the same geographic area. All subjects were healthy and appropriately physically active for their age, and none were involved in competitive sport activities. Their height and weight measurements were within the 3rd and 97th percentiles for age. Candidates were excluded if they had a history of chronic illness, if they had one or more fractures, or if they had taken any medication, hormones, vitamin preparations, or calcium supplements regularly.

All candidates for this study underwent a physical examination to obtain anthropometric measures and to assess pubertal development. Body weight was measured to the nearest 0.1 kg on a balance-beam scale (Seca, Hamburg, Germany), and height was measured to the nearest 1 mm by using a wall-mounted stadiometer (Holtain Ltd, Crosswell, United Kingdom). Body mass index was computed as weight/height² (in kg/m²). None of the patients had delayed pubertal development.

Informed consent was obtained from all of the parents or legal guardians of all the patients and volunteers. The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethical committee of our institution.

Study protocol

Concentrations of biochemical markers of bone turnover were measured in the patients at the time of diagnosis and after 2, 6, and 12 mo of a GFD. Bone mineral measurements were performed at the time of diagnosis and after 12 mo of treatment. Control subjects were studied only once for ethical reasons.

Biochemical measurements

Blood was allowed to clot immediately after venipuncture; serum was separated by centrifugation (1500 × g, 10 min, room temperature) and was stored at −30 °C until analyzed. Urine specimens were collected between 1000 and 1200 as the second voiding of the day to minimize the effect of the circadian rhythm of the excretion of collagen degradation products (16). Samples were aliquoted immediately and were stored at −30 °C until analyzed.

Intact parathyroid hormone concentrations were measured by an immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). The intraassay variance was <4%, and the interassay variance was <7%. The sensitivity of the assay was 1 pg/mL.

Bone-specific alkaline phosphatase (BALP) was measured in serum as a bone formation marker with the use of a commercial immunoassay (Alkphase-B; Metra Biosystems Inc, Mountain View, CA). Intraassay reproducibility was <4%, and interassay variation was <7%. The sensitivity of the assay was 0.7 U/L.

We measured urinary concentrations of N-terminal telopeptide of type I collagen (NTx) as a bone resorption index. NTx was measured by using an enzyme-immunosorbent assay (Osteomark; Ostex, Seattle). Assay values were standardized to an equivalent amount of bone collagen and were expressed in nanomoles bone collagen equivalents (BCE) per liter (nmol BCE/L). The sample results from a single urine collection were normalized for urine dilution by urinary creatinine analysis and were reported as nmol BCE/mmol creatinine. In our laboratory, the intraassay variation was <10%. The interassay precision was <9%, and sensitivity was 20 nmol BCE/L.

Urinary creatinine was measured by a colorimetric method (Creatinine; Sigma Diagnostics, St Louis). Serum concentrations of calcium, phosphorus, magnesium, and albumin were determined with the use of standard automated methods.
TABLE 3
Bone mineral content (BMC) measurements of patients with celiac disease at the time of diagnosis and after the first year of treatment and of healthy control children.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 22)</th>
<th>Healthy children (n = 428)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diagnosis</td>
<td>1 y</td>
</tr>
<tr>
<td>Spine BMC (g)</td>
<td>18.9 ± 2.3 (^2)</td>
<td>23.2 ± 2.6</td>
</tr>
<tr>
<td>Total-body BMC (g)</td>
<td>1179.5 ± 107.2 (^2)</td>
<td>1421.6 ± 114.9</td>
</tr>
</tbody>
</table>

\(^1\) \(\bar{x} \pm \text{SEM}\).
\(^2\) Significantly different from healthy children: \(^2 P = 0.032\), \(^3 P = 0.03\).

Bone mineral measurements

BMC was measured at the L2-L4 vertebrae level and in the whole skeleton. The data were analyzed with pediatric software (version 1.5e; Lunar Radiation Corp, Madison, WI). Bone mineral measurements were made with a dual-energy X-ray absorptiometer (DPX-L; Lunar Radiation Corp). The instrument was calibrated daily according to the manufacturer’s instructions. Reproducibility was calculated as the CV obtained by weekly measurements of a standard phantom and by repeated measurements obtained in 3 children of different ages. The CV for our instrument was 0.6% with the standard phantom; in vivo, we calculated a CV of 1.4% for the lumbar spine and 1.5% for the whole skeleton. The effective radiation dose for each scan was \(\approx 0.3 \mu\text{Sv}\) for the lumbar spine and \(< 0.03 \mu\text{Sv}\) for the whole-body scans (21).

Statistical analysis

Descriptive statistics were calculated for all the variables, and data are expressed as means \(\pm\) SEMs unless otherwise stated. All statistical analyses were conducted at the \(\alpha = 0.05\) level and were two-tailed. Distribution of the variables was checked by using the Shapiro-Wilk \(W\) test. The statistical software JMP IN (SAS Institute Inc, Cary, NC) was used for the analyses.

BALP and NTx were not normally distributed and therefore were log-transformed for statistical analyses. All other variables were normally distributed.

Analysis of variance for repeated measures was used to analyze the changes in the biochemical variables occurring during the follow-up period. Tukey-Kramer post hoc tests were performed to compare each time point.

Multiple regression analyses were performed to evaluate the differences between the patients and the control subjects after control for confounding variables. Bone metabolism indexes or bone mineral measurements were the dependent variables, whereas sex, age, and anthropometric measurements were the confounding variables; the presence of celiac disease was the independent variable. All anthropometric measurements were included initially, and the backward procedure was used to build the best model. Simple correlation analyses were performed to assess the relation between variables.

Finally, a one-group \(t\) test was used to compare the changes that occurred during treatment in bone mineral measurements with those expected, which were calculated in the control group as previously described (8). Briefly, annual BMC increments were obtained by using regression analyses with age as the independent variable.

RESULTS

Diagnosis

BMC measurements are shown in Table 2. The BMC of children with celiac disease was significantly lower than that of control children. After control for confounding variables, the BMC of the spine was on average 1.0 g (weighted difference) lower \((P = 0.032)\), and the BMC of the entire skeleton was on average 38.7 g (weighted difference) lower \((P = 0.03)\).

Serum concentrations of calcium, albumin, phosphorus, and magnesium and urinary concentrations of creatinine and calcium are shown in Table 3. All values were within normal limits. The mean serum intact parathyroid hormone concentration at the time of diagnosis was 30.8 ± 4.3 ng/mL (Table 3). None of the patients had elevated intact parathyroid hormone concentrations [normal range: 16.0–59.0 ng/mL (22)].

Serum BALP concentrations were significantly lower in patients with celiac disease at the time of diagnosis than in control subjects \((P = 0.0064; \text{Figure 1})\). The mean difference

TABLE 3
Serum and urinary measurements of calcium-related elements and of intact parathyroid hormone (iPTH) in patients with celiac disease at the time of diagnosis and during the first year of treatment.

<table>
<thead>
<tr>
<th></th>
<th>Diagnosis</th>
<th>2 mo</th>
<th>6 mo</th>
<th>1 y</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.54 ± 0.14</td>
<td>—</td>
<td>—</td>
<td>2.46 ± 0.02</td>
<td>2.20–2.70</td>
</tr>
<tr>
<td>Serum total protein (g/L)</td>
<td>62.7 ± 1.6</td>
<td>—</td>
<td>—</td>
<td>63.9 ± 0.9</td>
<td>60–80</td>
</tr>
<tr>
<td>Serum phosphorus (mmol/L)</td>
<td>1.97 ± 0.24</td>
<td>—</td>
<td>—</td>
<td>1.68 ± 0.05</td>
<td>0.95–1.75</td>
</tr>
<tr>
<td>Serum magnesium (mmol/L)</td>
<td>0.84 ± 0.04</td>
<td>—</td>
<td>—</td>
<td>0.80 ± 0.02</td>
<td>0.7–1.0</td>
</tr>
<tr>
<td>Urinary creatinine (mg/dL)</td>
<td>108.1 ± 13.8</td>
<td>—</td>
<td>—</td>
<td>72.4 ± 9.0</td>
<td>60–180</td>
</tr>
<tr>
<td>Urinary calcium (mmol/L)</td>
<td>1.51 ± 0.23</td>
<td>—</td>
<td>—</td>
<td>2.70 ± 0.5</td>
<td>2.1–2.8</td>
</tr>
<tr>
<td>Serum iPTH (ng/mL)</td>
<td>30.8 ± 4.3</td>
<td>34.0 ± 3.7</td>
<td>27.5 ± 2.7</td>
<td>25.9 ± 2.4</td>
<td>16.0–59.0</td>
</tr>
</tbody>
</table>

\(^1\) \(\bar{x} \pm \text{SEM}\).
between the 2 groups, after correction for confounding variables, was 14.8 ± 5.2 U/L. Conversely, urinary concentrations of NTx were significantly higher in patients with celiac disease than in healthy control subjects (P < 0.0001; Figure 2). The difference between the 2 groups was on average 126.5 ± 22.8 nmol BCE/mmol creatinine. Correlations between bone and metabolism variables, anthropometric measurements, and bone mineral measurements are shown in Table 4.

Gluten-free diet

After the first year of treatment, BMC values did not differ significantly between the 2 groups of children. The weighted
The bone metabolism rate can be assessed noninvasively by measurement in serum and urine of specific markers of bone formation and bone resorption. In the current study, we measured the bone fraction of alkaline phosphatase as a bone formation marker and urinary NTx as a bone resorption index. We found very low BALP values in untreated patients, indicating an impairment of osteoblastic activity. Similar findings were previously reported in a small group of children with celiac disease at the time of diagnosis (18). In that study, bone formation was assessed by serum measurements of osteocalcin and the carboxyl terminal propeptide of type I collagen, and untreated children had significantly lower values for both markers than did healthy control subjects. Unfortunately, no markers of bone resorption were measured. In the present study, the bone resorption rate of the patients was extremely high before treatment. The combined effect of a low bone formation rate and enhanced bone resorption may explain the low BMC values found at diagnosis in our patients.

During treatment, our patients showed a marked and constant increase in serum BALP concentrations. The increment was remarkable even after only 2 mo of the GFD, illustrating the prompt response of bone-forming cells to treatment. Similar responses were documented in a previous study, which showed remarkable increments in bone formation markers after 1 and 3 mo of a GFD (18). In our patients, however, the effect of treatment was not as impressive on the bone resorption rate: NTx concentrations did not change significantly during follow-up. Moreover, serum BALP concentrations did not differ significantly from those of control subjects after 6 mo of treatment, whereas NTx concentrations never normalized during the follow-up period. Similar findings were reported after 4 y of a GFD (8); patients with celiac disease had serum BALP and urinary NTx concentrations that were markedly higher than those in control subjects.

The reason for the high concentrations of bone metabolism indexes is still unclear. This phenomenon may be a sign of active growth, which occurs immediately after the initiation of a GFD and which is not yet complete even after long-term treatment (23, 24). Another hypothesis is the presence of yet unknown factors that disrupt the normal regulation of bone remodeling units. Nevertheless, the beneficial effect of a GFD on bone metabolism was confirmed in a study that reported the results of a gluten challenge in children with celiac disease (25). In that study, after 1 mo of gluten exposure, serum BALP

### DISCUSSION

Bone mineral alterations were previously documented at the time of diagnosis in children with celiac disease (2–4). Both cross-sectional and longitudinal studies have shown variable, although remarkable, improvement in bone mass during consumption of a GFD (2–10). In the present study, we confirmed these previous results. We measured BMC at the lumbar spine and in the whole skeleton and found that the mineral deficit present at the time of diagnosis was completely corrected after 1 y of treatment. However, the biological mechanisms that favor the reduced bone mineralization and that are responsible for the rapid improvement observed have been sparsely studied.
concentrations decreased significantly in all patients, indicating depressed osteoblastic activity.

Our data differ substantially from those reported in studies of untreated adult patients. High serum concentrations of bone formation indexes have been found at the time of diagnosis of celiac disease (26–28), coupled with a high bone resorption rate (26, 27). Conversely, treated patients showed lower values for bone formation (26–28) and bone resorption (26, 27) markers. The higher concentrations of bone metabolism markers observed at the time of diagnosis in adult patients has been attributed to a relative state of hyperparathyroidism, which is corrected by dietary treatment (26–28). To the contrary, our patients had serum concentrations of parathyroid hormone within the reference range at the time of diagnosis and during the follow-up period. These discrepancies between adults and youths with celiac disease are not surprising and are probably due to the different metabolism rates that characterize the diverse periods of life. During growth, bone formation and resorption are mainly devoted to the changes in the shape and dimensions of bones called modeling. During adulthood, the bone metabolism rate is much lower and is mainly aimed at maintaining good quality and efficient bone tissue (bone remodeling).

Another possible explanation for the difference is the different role of malabsorption in adults and youths. The diet of an average adult is usually poorer in calcium than are the diets of children. Therefore, impaired intestinal absorption of calcium in adults may lead to hypocalcemia, which is promptly corrected by an increased secretion of parathyroid hormone, which ultimately leads to loss of bone mineral. Higher availability of calcium in younger patients may prevent hypocalcemia and may therefore explain the normal parathyroid hormone concentrations found in our patients. Nevertheless, the primary mechanism of low bone mineral in youths with celiac disease remains unknown. Some evidence suggests a possible role of interleukins and autoantibodies in the genesis of osteopenia (29, 30). These elements might be more relevant in the developing skeleton than are nutritional factors.

In summary, the bone metabolism rate, as measured by specific biochemical markers, is altered in untreated children with celiac disease. The alterations (low bone formation and enhanced bone resorption) may be the cause of the osteopeny observed in children with celiac disease. Remarkable changes occur after the initiation of a GFD, which result in a more balanced equilibrium despite a partial normalization of biochemical bone metabolism markers.

GB participated in the study design, data collection (recruitment of patients and clinical data collection) and interpretation, and manuscript preparation. SB contributed to the data collection (bone density measurements, serum and urine collection, and anthropometric measurements) and interpretation. MCP performed all biochemical analyses and participated in the data interpretation. SM participated in the study design, data collection and interpretation, data analysis, and writing of the manuscript. None of the authors had a conflict of interest.

REFERENCES