Abnormal Skeletal Strength and Microarchitecture in Women With Celiac Disease

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Context: Osteoporosis is often a presenting sign of celiac disease (CD). Whether skeletal fragility in CD is associated with microarchitectural abnormalities is not known.

Objective: The objective of the study was to evaluate microarchitecture and biomechanical properties of bone in CD.

Design: This was a case-control study.

Setting: The study was conducted at a university hospital outpatient facility.

Patients: Patients included premenopausal women with newly diagnosed CD (n = 33) and healthy controls (n = 33).

Main Outcome Measures: Areal bone mineral density by dual-energy x-ray absorptiometry was measured as was trabecular and cortical volumetric bone mineral density (vBMD) and microarchitecture by high-resolution peripheral computed tomography of the distal radius and tibia. Whole-bone stiffness estimated by finite element analysis. PTH, 25-hydroxyvitamin D, and bone turnover markers were also measured.

Results: Groups had similar age, race, and body mass index. Both groups had sufficient 25-hydroxyvitamin D and normal calcium and PTH. Areal bone mineral density was lower in CD. By high-resolution peripheral computed tomography, CD had lower trabecular vBMD, fewer, more widely, and irregularly spaced trabeculae at both the radius and tibia (8%–33%). At the tibia, they also had lower total density (8%) and thinner cortices (10%). Whole-bone stiffness and failure load were lower (11%–21%) in CD at both sites. Biomechanical deficits were associated with trabecular abnormalities.

Conclusions: Women with CD had abnormal vBMD and microarchitecture at both the radius and tibia. Trabecular bone was preferentially affected. These deficits were associated with lower estimates of skeletal strength. These findings suggest a potential structural mechanism for skeletal fragility in CD and support further research into the pathogenesis of fracture in this population.

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Celiac disease (CD) is a chronic intestinal disorder affecting approximately 1% of the population of Europe and the United States (1). Patients with CD have an immune reaction to the gliadin fraction of gluten, a protein found in barley, wheat, and rye, the ingestion of which leads to villous atrophy and chronic inflammation of the small bowel mucosa (2, 3). CD can have many extraintestinal manifestations, including osteomalacia, and osteoporosis. Fractures are often a presenting sign of CD. Low bone mineral density (BMD) has been reported in many studies of patients with CD, with estimates as high as 70%, depending on the age, sex, menopausal status, and the general health of the population studied (4–8). Although many studies have not been designed to look at fracture rates, some epidemiological studies (9–17) and a recent meta-analysis (18) have demonstrated an increased risk of fractures in patients with CD. More recently a population-based study demonstrated that persistent villous atrophy was associated with an increased risk of hip fracture, despite a gluten-free diet (19).

Abnormal bone metabolism in patients with CD is multifactorial. Low BMD in celiac patients has been directly related to severity of histological disease by intestinal biopsy, providing further support for the role of malabsorption and inflammation as factors in the development of bone disease (20). Malabsorption of calcium, magnesium, and vitamin D resulting from villous atrophy can result in secondary hyperparathyroidism (21–23). Patients often have concurrent lactose intolerance, which can also contribute to inadequate calcium and vitamin D intake. Increased inflammatory cytokines, IL-1, IL-6, TNFα, and receptor activator of nuclear factor-κB ligand in CD patients may contribute to increased osteoclast activity, decreased osteoblast activity, and uncoupled bone turnover (24–26). Other factors that may contribute to fragility and fracture risk in CD patients include zinc deficiency and low IGF-1, low body mass index (BMI), malnutrition and hypogonadism, and autoantibodies (3, 27). These factors have the potential to increase fragility in various ways and may have differential effects on the cortical and trabecular compartments of bone. These disparate effects cannot be captured by dual-energy x-ray absorptiometry (DXA), the standard tool for assessment of BMD. Furthermore, DXA is affected by bone size and may be artifactualy low in CD patients who were ill during adolescence and thus may not have attained peak bone mass or predicted adult height.

The availability of high-resolution peripheral quantitative computed tomography (HR-pQCT) has provided us with the ability to measure true volumetric BMD (vBMD) in patients with CD and to study the differential effects of CD on cortical and trabecular bone. This technology, with an isotropic voxel size of 82 μm permits noninvasive measurement of vBMD and trabecular and cortical microarchitecture. In addition, data sets from individual HR-pQCT scans can be computationally modeled by microstructural finite element analysis to assess bone stiffness and failure load, surrogate measures of strength. In recent years, this technology has become an important tool for investigation of microstructural and biomechanical mechanisms of fragility in many populations (28–35). In this study, we compared premenopausal women with CD with premenopausal controls using standard DXA and HR-pQCT. We hypothesized that women with CD would have lower vBMD and worse microarchitecture compared with controls. We further hypothesized that cortical bone would be preferentially affected due to the effects of secondary hyperparathyroidism.

Materials and Methods

Participants

Premenopausal women with CD (n = 33), between the ages of 20 and 50 years, were recruited at Columbia University Medical Center by physician referral. The CD diagnosis was made on the basis of serology and small bowel biopsy. Histology was not available in four of the CD subjects whose biopsies had been performed at outside institutions. Women were included if they were newly diagnosed with celiac disease and were untreated. Subjects who had been following a gluten-free diet for more than a month were excluded. Control subjects (n = 33) were healthy premenopausal women who had negative serological antibody tests for CD. Controls were recruited as part of another ongoing study that mandated they have normal bone density (Z-score ≥ −1) and no medical conditions associated with osteoporosis including endocrinopathies (eg, untreated hypothyroidism, Cushing’s syndrome, or prolactin-secreting pituitary adenoma), renal insufficiency, liver disease, or medication exposures that could affect bone metabolism (eg, glucocorticoids, anticonvulsants, anticoagulants, methotrexate, and aromatase inhibitors).

All subjects provided written informed consent, and the Institutional Review Board of Columbia University Medical Center approved this study. At the study visit, past medical history and medication use were assessed with questionnaires. A physical examination was performed including height by Harpenden stadiometer and weight, and BMI was calculated. Fasting blood samples were obtained.

Areal bone mineral density (aBMD)

aBMD was measured by DXA (QDR-4500; Hologic Inc) at the lumbar spine L1-L4 (LS), total hip (TH), femoral neck (FN), ultradistal radius (UDR), and the one third radius (1/3R).

High-resolution peripheral quantitative computed tomography

HR-pQCT (XtremeCT) was performed by immobilizing the nondominant forearm and the ipsilateral tibia in a carbon fiber shell and scanning as previously described (28–30). The machine provided measures of volumetric bone density, bone size, and three-dimensional quantitative in vivo measurements of the mi-
croarchitectural structures. The European Forearm Phantom was scanned daily for quality control. All scans were acquired by the same highly experienced technician. The HR-pQCT analysis methods used in this study have described and validated in several of our previously published studies (28–30). In addition to standard cortical and trabecular measurements of vBMD, cortical thickness, and trabecular microarchitecture, HR-pQCT data were computationally modeled using microfinite element analysis to calculate whole-bone stiffness and failure load, measures of bone’s resistance to force. To evaluate the cortical bone structure, a validated autosegmentation method was applied to separate the cortical and trabecular compartments and measure cortical porosity (percentage). Cortical porosity was calculated as the percentage of void space in the cortex. This method, distributed by the manufacturer (Scanco Medical), has been validated for accuracy and reproducibility (36).

Biochemical measurements
Serum 25-hydroxyvitamin D (25OHD) was measured by ultra-performance liquid chromatography combined with tandem mass spectrometry using a 1290 UPLC and a 6410 tandem mass spectrometer (Agilent). Interassay coefficient of variation (CV) is 2.9% for 25OHD3 and 5.4% for 25OHD2. Serum 1,25-dihydroxyvitamin D was measured by RIA (Diasorin; CV 12%). Intact PTH was measured by immunoradiometric assay (Scantibodies Laboratories; CV 6.8%). Serum C-telopeptide (CTX) was measured by an ELISA (Quidel; CV 8%). Serum was archived at −80°C and analyzed in one batch after all visits were completed.

Statistical methods
All analyses were performed using SAS version 9.3 (SAS Institute). Group means were compared by independent Student’s t test for continuous measures or a Fisher’s exact test for categorical indices. The percentage difference in measures of celiac patients relative to controls was calculated as a difference between group means with the SE of the difference (see Figures 2 and 4). Two-sided values of P < .05 were considered to indicate statistical significance. No penalty for multiple comparisons was applied.

Results
Sixty-six premenopausal women were enrolled (mean age 33 ± 8 y), 33 with CD and 33 controls. Group characteristics are detailed in Table 1. Women with CD and controls were of similar age and BMI. Use of calcium supplements was greater among celiac subjects, accounting for a daily calcium intake twice that of controls (1278 ± 664 vs 595 ± 120 mg/d; P < .01). Similarly, use of vitamin D supplements was twice as great among patients with CD compared with controls. Vitamin D intake varied widely, with five celiac subjects using high-dose vitamin D supplementation (>1600 IU daily). Average daily dose of vitamin D was 1400 ± 1977 IU among CD patients and 475 ± 96 IU among controls (P = .37). Duration of supplementation among CD patients was also quite variable, between 2 weeks and 5 years (average 17 ± 23 mo). There were no fractures in the control group. Among the women with celiac disease, 5 of 33 had a history of fracture. Fracture sites included the tibia, wrist, and clavicle. Three subjects had metatarsal stress fractures.

Serum 25-hydroxyvitamin D was in the sufficient range in both groups but substantially higher in celiac subjects than in controls (51%; P < .01). Similarly, use of vitamin D supplements among CD patients was also quite variable, between 2 weeks and 5 years (average 17 ± 23 mo). There were no fractures in the control group. Among the women with celiac disease, 5 of 33 had a history of fracture. Fracture sites included the tibia, wrist, and clavicle. Three subjects had metatarsal stress fractures.

Table 1. Characteristics of the Study Population
(Mean ± SD)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Celiac (n = 33)</th>
<th>Control (n = 33)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>33 ± 8</td>
<td>33 ± 9</td>
<td>.88</td>
</tr>
<tr>
<td>Race, % white</td>
<td>100%</td>
<td>100%</td>
<td>n/a</td>
</tr>
<tr>
<td>Height, cm</td>
<td>164 ± 7</td>
<td>165 ± 7</td>
<td>.03</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65 ± 14</td>
<td>68 ± 15</td>
<td>.56</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24 ± 5</td>
<td>25 ± 5</td>
<td>.58</td>
</tr>
<tr>
<td>Use of calcium</td>
<td>36%</td>
<td>21%</td>
<td>.29</td>
</tr>
<tr>
<td>supplements, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium supplements, total daily dose, mg</td>
<td>1278 ± 664</td>
<td>595 ± 120</td>
<td>.01</td>
</tr>
<tr>
<td>Use of vitamin D supplements, %</td>
<td>67%</td>
<td>33%</td>
<td>.02</td>
</tr>
<tr>
<td>Vitamin D supplements, total daily dose, IU</td>
<td>1399 ± 1977</td>
<td>475 ± 96</td>
<td>.37</td>
</tr>
</tbody>
</table>

Abbreviation: n/a, not available.

Table 2. Biochemistries in Celiac and Control Subjects
(Mean ± SD)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Celiac (Mean ± SD)</th>
<th>Control (Mean ± SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (8.6–10.2 mg/dL)</td>
<td>9.0 ± 0.1</td>
<td>9.3 ± 0.1</td>
<td>.02</td>
</tr>
<tr>
<td>25OHD (30–80 ng/mL)</td>
<td>42.7 ± 16.1</td>
<td>32.9 ± 13.9</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>1,25(OH)2D (25–66 pg/mL)</td>
<td>74.9 ± 35.1</td>
<td>48.8 ± 10.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PTH (14–66 pg/mL)</td>
<td>29.5 ± 12.5</td>
<td>27.4 ± 9.8</td>
<td>.92</td>
</tr>
<tr>
<td>BSAP (11.6–42.7 U/L)</td>
<td>24.3 ± 10.4</td>
<td>21.6 ± 7.0</td>
<td>.30</td>
</tr>
<tr>
<td>OC (8.4–33.9 ng/mL)</td>
<td>17.0 ± 6.8</td>
<td>19.4 ± 8.2</td>
<td>.26</td>
</tr>
<tr>
<td>P1NP (16–96 µg/L)</td>
<td>48.9 ± 15.0</td>
<td>55.0 ± 22.9</td>
<td>.26</td>
</tr>
<tr>
<td>CTX (0.11–0.74 ng/mL)</td>
<td>0.38 ± 0.18</td>
<td>0.40 ± 0.25</td>
<td>.80</td>
</tr>
</tbody>
</table>

Abbreviations: OC, osteocalcin; 1,25(OH)2D, 1,25-dihydroxyvitamin D.
Bone size, vBMD, and cortical and trabecular microarchitecture were assessed by HR-pQCT (Figure 2). At the radius, the total cross-sectional area was smaller in celiac subjects (−8.4%; P < .02). Both cortical (−8.9%; P = .07) and trabecular (−8.3%; P = .07) compartments tended to be smaller in celiac subjects. Trabecular indices were worse in celiac subjects, who had lower trabecular density (−16.9%; P < .009) and number (−10.9%; P < .007) and greater trabecular separation (18.4%; P < .007) and network heterogeneity (32.6%; P < .02). Trabecular thickness tended to be lower in celiac subjects (−8.2%; P = .07). In contrast, total and cortical density and cortical thickness did not significantly differ.

At the tibia, total cross-sectional and trabecular areas were similar between groups, whereas the cortical area thickness tended to be lower in celiac subjects (−12.7%; P < .002). Total density tended to be lower in celiac subjects (−8.3%; P = .053). Cortical density was similar, but cortical thickness was lower in celiac subjects (−10.4%; P < .03). As at the radius, trabecular abnormalities were observed in celiac subjects, with lower trabecular density (−11.3%; P < .04) and number (−9.4; P < .02) and greater separation and network heterogeneity (13.0%; P < .02, and 17.8%; P < .02, respectively). Trabecular thickness did not differ. Cortical porosity was significantly higher in celiac subjects compared with the controls at the radius (celiac: 1.6% ± 1.0% vs control: 0.9% ± 0.5%; P < .001) but did not differ at the tibia (celiac: 2.9% ± 1.5% vs control 2.9% ± 1.6%; P = .83). Representative HR-pQCT scans of the radius and tibia in a CD subject and a control of similar age and BMI are shown in Figure 3.

Biomechanical properties of bone were assessed by microstructural finite element analysis (Figure 4). Celiac subjects had lower whole-bone stiffness at both the radius (−15.2%; P < .002) and tibia (−12.6%; P < .007). Similarly, failure load was lower in celiac patients at both the radius (−20.5%; P < .01) and tibia (−10.5%; P < .03). Lower stiffness and failure load at the radius were directly associated with worse microarchitectural parameters. The strongest associations were with trabecular density (r = 0.69; P < .0001 for both stiffness and failure load) and trabecular separation (r = −0.58; P < .001 for association with stiffness; r = −0.60; P < .0006 for association with failure load).

To determine whether vitamin D supplementation by the CD subjects impacted the structural results, we compared CD subjects who were (n = 22) and were not (n = 11) using vitamin D. There was no difference in aBMD at the spine, hip, or FN, although aBMD at the 1/3R was lower in the group on vitamin D supplementation (P < .05). There was no difference between patients who were and were not using vitamin D supplements in vBMD or any microarchitectural variable.

**Discussion**

This study demonstrated for the first time that premenopausal women with celiac disease (gray bars) and matched controls (striped bars), with lower trabecular density (15.2%; P < .002) and trabecular separation (18.4%; P < .007). Lower stiffness and failure load at the radius were directly associated with worse microarchitectural parameters. The strongest associations were with trabecular density (r = 0.69; P < .0001 for both stiffness and failure load) and trabecular separation (r = −0.58; P < .001 for association with stiffness; r = −0.60; P < .0006 for association with failure load).
with celiac disease have lower vBMD, abnormal micro-
architecture, and lower strength, measured as stiffness and
failure load compared with normal premenopausal
women of similar age, BMI, gender, and race/ethnicity. In
counter to our hypothesis that cortical abnormalities
would predominate, we found pronounced deficits in tra-
becular bone, which were associated with impaired bio-
mechanical properties. In accordance with prior litera-
ture, we found that aBMD by DXA was also lower in CD
patients.

Although we hypothesized that cortical bone would be
preferentially affected as a result of secondary hyperpara-
thyroidism, we did not find this to be the case. Many other
groups have reported that vitamin D deficiency and sec-
ondary hyperparathyroidism are common in CD (23, 37,
38). Patients with secondary hyperparathyroidism have
lower BMD than those with normal PTH and less
improvement in BMD after initiation of gluten-free diet (39).
In contrast, our patients with CD did not have secondary
hyperparathyroidism. They had normal serum calcium
and sufficient 25OHD levels, possibly because most of our
CD patients were taking high doses of calcium and vitamin
D. Some of these patients had been diagnosed with vitamin
D deficiency in the past and started on treatment before the
diagnosis of CD was established, which likely influenced
our results.

It is noteworthy that despite substantially higher cal-
cium intake, serum calcium was lower in CD patients com-
pared with controls, suggesting that these patients did
have calcium malabsorption. Measurements of 24-hour
urine samples, which we do not have, would have been
helpful in making that assessment. Lower serum calcium
may also have reflected active skeletal remineralization in CD patients.

Serum 25OHD was greater among CD patients and was likely the
primary influence governing PTH in this group. Several of the CD patients
were using high doses of vitamin D at the time of the study evaluation.
Mean vitamin D intake was substantially higher among CD patients
compared with controls, although this difference was not significant
due to the large variability in vitamin D dose. The cross-sectional nature of
this study precludes our assessment of past abnormalities in calcitropic
hormones, which may have significantly influenced skeletal structure
and strength, particularly if the abnormal calcitropic hormones were
longstanding.

We found substantial abnormalities in trabecular bone
and associated biomechanical compromise despite normal
PTH and preserved cortical bone in CD patients. Although
recent evidence has demonstrated abnormalities in both
cortical and trabecular microarchitecture in patients with
primary hyperparathyroidism (40), the preferential effect
on trabecular bone suggests that other factors may play a
role in fragility in this population. Other potential mech-
anisms that could preferentially affect trabecular bone be-
because of its increased metabolic activity include uncoupled
bone turnover related to increased inflammatory cyto-
kines and low IGF-1 (3, 24). Autoimmune factors may also
play a role, including direct effects of tissue transglutami-
nase antibodies on bone (27). The role of all of these fac-
tors warrants further investigation.

Compared with controls, premenopausal women with
CD had lower trabecular density and fewer, more widely
and irregularly spaced trabeculae. These abnormalities
were directly associated with lower stiffness and failure
load. In contrast, cortical abnormalities were more vari-
able and did not appear to play as significant a role in
biomechanical compromise in this group. Cortical poros-
ity was higher at the radius in CD patients but did not
differ at the tibia. Cortical area was smaller and thickness
was lower compared with controls at the tibia, but we
found no difference in cortical density at either site. A
study using an older, lower resolution peripheral com-
puted tomography scanner (unable to assess bone micro-
architecture) found that women with CD had lower total
and cortical vBMD and smaller cortical cross-sectional
area as well as lower trabecular vBMD compared with

Figure 4. Comparison of percentage difference ± SEM in whole-bone stiffness and failure load in celiac and control subjects at the radius (striped bars) and tibia (black bars), presented as percentage difference. *, P < .05; **, P < .01.
controls (37). Bone strength estimated by cross-sectional moment of inertia and bending strength index was lower. Most subjects in that study had vitamin D deficiency and secondary hyperparathyroidism, which may have accounted for the observed abnormalities in cortical parameters (37). After treatment with a gluten-free diet, calcium and 25OHD increased and PTH decreased; vBMD and estimates of strength increased as well. Men in this study did not have the same abnormalities or response to treatment, suggesting that there may be differences in the extent of skeletal disease in CD according to sex and providing support for studying the groups separately.

We found that aBMD by DXA was lower in women with CD compared with controls. This finding was universal at all skeletal sites. Our results are consistent with other studies finding lower aBMD in newly diagnosed celiac patients compared with healthy controls (4–8, 21). Patients in many of these studies had vitamin D and calcium deficiency and secondary hyperparathyroidism, whereas we detected lower aBMD in the absence of these derangements. It is conceivable that the abnormalities we observed in BMD reflect past derangements in PTH, calcium, and vitamin D. In addition, CD patients did have lower cross-sectional area at the radius by HR-pQCT, and smaller bone size may have contributed to lower aBMD values by DXA.

This study had both unique strengths and limitations. To our knowledge this is the first study to evaluate the relationship between vBMD, microarchitecture, and strength in patients with CD. Our sample was restricted to premenopausal women, which allowed us to investigate the relationship between CD and bone without the influence of differences in sex or menopausal status. Furthermore, groups were matched by race and of similar age and BMI. Limitations of the study include the small size and cross-sectional design. Although our subjects had normal calcium, vitamin D, and PTH at the time of evaluation, it is not possible to ascertain whether prior derangements in calcitropic hormones resulted in the observed densitometric and microstructural abnormalities. It is conceivable that CD patients had abnormalities in skeletal mineralization, not detected by our techniques. Increased osteoid may have contributed to lower areal and volumetric BMD. Threshold-based measurements, such as estimated bone stiffness, would likely not account for increased osteoid. Another drawback is that our controls had been recruited for another study that required normal BMD for inclusion. We might not have detected as robust differences if the selection of controls had been population based and there were no BMD criterion for entry. However, this cutoff also ensured that we selected healthy controls and not women with an undiagnosed cause of secondary osteoporosis. The controls were also slightly but significantly taller than CD women, which may have contributed to larger bone size, and may have masked potential differences in cortical thickness. As mentioned earlier, there was some selection bias in our CD group because these were women primarily referred from the celiac practice at Columbia University Medical Center. Those who were willing to participate may have had more concerns about their bone health than the general population, as evidenced by their high intakes of calcium and vitamin D.

In conclusion, we found premenopausal women with CD had lower bone strength than controls matched for race and of similar age and BMI. Biomechanical deficits were directly related to abnormalities in trabecular bone vBMD and microarchitecture. These deficits existed despite sufficient calcium and 25OHD and normal PTH at the time of evaluation. These findings suggest a potential structural mechanism for skeletal fragility in CD and lay the foundation for further research into the pathogenesis of fracture in this population.

Acknowledgments

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