Histomorphometric Analysis Reveals Reduced Bone Mass and Bone Formation in Patients With Quiescent Crohn's Disease

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See related article, Cottone M et al, on page 30 in *CGH*; see editorial on page 22.

BACKGROUND & AIMS: Crohn's disease (CD) is associated with an increased prevalence of osteoporosis, but the pathogenesis of this bone loss is only partly understood. We assessed bone structure and remodeling at the tissue level in patients with quiescent CD. We also investigated the roles of osteocyte density and apoptosis in CD-associated bone loss. **METHODS:** The study included 23 patients with quiescent CD; this was a subgroup of patients from a large randomized, double-blind, placebo-controlled, multicenter trial. We obtained transiliac bone biopsy samples and performed histomorphometric analysis. Results were compared with data from age- and sex-matched healthy individuals (controls). **RESULTS:** Trabecular bone volume was decreased among patients with CD compared with controls (18.90% vs 25.49%; $P < .001$). The low bone volume was characterized by decreased trabecular thickness (120.61 vs 151.42 μm; $P < .01$). Bone formation and resorption were reduced, as indicated by a decreased mineral apposition rate (0.671 vs 0.746 μm/day; $P < .01$) and a low osteoclast number and surface area compared with controls and published values, respectively. In trabecular bone of patients with CD, osteocyte density and apoptosis were normal. The percentage of empty lacunae among patients was higher than that of published values in controls. **CONCLUSIONS: In adult patients with quiescent CD, bone histomorphometric analysis revealed a reduction in bone mass that was characterized by trabecular thinning. The CD-associated bone loss was caused by reduced bone formation, possibly as a consequence of decreased osteocyte viability in the patients' past.**

Keywords: Bone Histomorphometry; Osteocyte Apoptosis; Inflammatory Bowel Disease; Osteoporosis.

Patients with inflammatory bowel disease (IBD), in particular patients with Crohn's disease (CD), are at increased risk for bone loss. The prevalence of osteopenia and osteoporosis in patients with IBD varies between studies and is reported to be up to 42% and 16%, respectively.[1] The pathogenesis of bone loss in patients with IBD is complex, multifactorial, and only partly understood. General risk factors such as malnutrition, malabsorption, calcium and vitamin D deficiency, and corticosteroid treatment are known to be involved.[2] However, these factors cannot completely explain the bone loss. Recently, the inflammatory process itself has been suggested to play a pivotal role in IBD-associated bone loss. For example, proinflammatory cytokines that contribute to the intestinal immune response in IBD, such as tumor necrosis factor α and factors belonging to the receptor activator for nuclear factor κB ligand (RANKL)/osteoprotegerin system, are known to enhance bone resorption.[3,4]

Bone remodeling involves a balanced process of bone resorption by osteoclasts and subsequent bone formation by osteoblasts. An imbalance in this process can be assessed by measurement of biochemical markers of bone turnover. In patients with IBD, bone resorption is increased, as indicated by elevated levels of the markers deoxypyridinoline and cross-linked N-telopeptide of type 1 collagen.[5–7] Bone formation in patients with IBD is less well characterized. Increased levels of (bone-specific) alkaline phosphatase,[7] decreased levels of osteocalcin,[7–9] and normal levels of bone formation markers have been reported in patients with IBD.[10] Therefore, based on biochemical markers of bone turnover, the bone remod-

Abbreviations used in this paper: 25(OH)D, 25-hydroxyvitamin D_3; ALP, alkaline phosphatase; CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; IBD, inflammatory bowel disease; RANKL, receptor activator for nuclear factor kappa B ligand; TNF, tumor necrosis factor.

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eling balance in patients with IBD is characterized by a net increase of bone resorption.

At the tissue and cellular level, low bone mass is caused by a disturbed regulation of bone remodeling as well.[11] Histomorphometric analysis of bone biopsy specimens is a powerful tool to show disturbances in bone remodeling. In patients with IBD, there are only few data concerning bone histomorphometry. Croucher et al showed reduced bone formation and a mild mineralization defect in patients with IBD who had osteoporosis.[12] Ward et al observed both a suppressed bone formation and bone resorption in children with newly diagnosed IBD,[13] whereas Hessov et al reported normal bone remodeling and mineralization in young patients with CD.[14]

The regulation of bone remodeling is believed to be supervised by osteocytes, the third cell type in bone in addition to osteoblasts and osteoclasts. As a consequence, a lack of functionally active osteocytes might underlie bone pathology. In fact, the number of osteocytes appears to decline with aging[15] and is lower in postmenopausal women, especially in those with a vertebral fracture, when compared with premenopausal women.[16,17] Moreover, the percentage of apoptotic osteocytes in postmenopausal women has been shown to be associated with the level of bone remodeling.[18] Information on osteocyte density and osteocyte function in bone of patients with IBD is lacking thus far.

To ascertain a better understanding of bone biology in patients with IBD, the aims of this study were to (1) assess bone structure and remodeling at the tissue level in patients with quiescent CD in comparison with healthy controls and (2) investigate whether osteocyte density and osteocyte apoptosis are related to bone structure and remodeling parameters.

Patients and Methods

Patients

Twenty-three patients with quiescent CD participated in this study. These patients were a subgroup included in a large randomized, double-blind, placebo-controlled, multicenter trial on the effect of risedronate in patients with quiescent CD who had osteopenia (N = 131, Crohn and Bone Study). The current study describes data obtained from patients at baseline. Patients were diagnosed with CD using clinical, endoscopic, histologic, and radiologic criteria according to Lennard-Jones.[19] Patients had to be in remission (Crohn's Disease Activity Index [CDAI] <150). Although 2 patients had a CDAI >150, these patients had absence of symptoms of active disease and no signs of active inflammation on sigmoidoscopy and as such were defined to be in clinical remission. Another inclusion criterion was a lumbar spine and/or total hip bone mineral density with a T-score of −1 to −2.5 SD (osteopenia). Osteopenia is the most prevalent state of bone loss in usually relatively young patients with CD and is believed to progress to (presenile) osteoporosis. Hence, as such it is the most relevant population to study. Of the 23 patients included, 12 were women, of whom one was postmenopausal. Patients with current or recent bisphosphonate or corticosteroid treatment (<1 year and <3 months, respectively, before inclusion) were excluded, as well as patients with known metabolic bone diseases and serum levels of 25-hydroxyvitamin D_3 less than 25 nmol/L. The study was approved by the institutional review board at each participating medical center. All patients gave written informed consent.

Biochemistry

Biochemical measurements in serum included calcium (colorimetric assay; Roche Diagnostics, Mannheim, Germany), 25-hydroxyvitamin D_3 (radioimmunoassay; DiaSorin, Stillwater, MN), C-reactive protein (immunoturbidimetric assay; Roche Diagnostics), and total alkaline phosphatase (International Federation of Clinical Chemistry method; Roche Diagnostics). Intra-assay and inter-assay coefficients of variation of the assays were as follows: calcium, 0.9% and 1.3%; 25-hydroxyvitamin D_3, 8% and 10%; C-reactive protein, 1.2% and 1.4%; and alkaline phosphatase, 0.7% and 2.4%. Measurements were performed by the laboratories of Clinical Chemistry and Endocrinology of the VU University Medical Center.

Bone Biopsies

Patients received 2 doses of tetracycline (250 mg 4 times daily) for 2 days separated by an interval of 10 days. Three to 7 days after the last dose, transiliac bone biopsy specimens were taken using Bordier's trephine (ID, 8 mm). The bone biopsy specimens were fixed in cold 4% phosphate-buffered formaldehyde, dehydrated in graded ethanol, and embedded in 80% methylmethacrylate (BDH Chemicals, Poole, England) supplemented with 20% dibuthylphtalate (Merck, Darmstadt, Germany), 8 g/L lucidol CH-50L (Akzo Nobel, Deventer, The Netherlands), and 22 μL/10 mL *N,N* dimethyl-p-toluidine (Merck). Five-micrometer-thick undecalcified sections were cut with a Polycut 2500 S microtome (Reichert–Jung, Nussloch, Germany). Goldner's trichrome stain was performed to distinguish between calcified bone and noncalcified matrix (osteoid). Tartrate-resistant acid phosphatase stain was used to visualize osteoclasts. Unstained sections were used for fluorescence microscopy to measure tetracycline labels.

Immunohistochemistry

Apoptotic cells were visualized by immunohistochemical detection of activated caspase-3. Immunohistochemistry was performed on two 5-μm sections of each biopsy specimen, which were obtained with an interval of 30 μm. Sections were cut and transferred to poly-L-lysine–coated slides. After deplastification and rehydration, sections were decalcified for 10 minutes with 1%

acetic acid. Antigen retrieval was performed by a 30-minute incubation with 0.5% saponin (Sigma, St Louis, MO) in phosphate-buffered saline (PBS) and a 10-minute incubation with 3.5 μg/mL deoxyribonuclease II (Sigma) in 25 mmol/L Tris plus 10 mmol/L magnesium sulfate. Sections were incubated with 3% hydrogen peroxide in methanol to block endogenous peroxidase and with 5% normal goat serum in PBS plus 0.05% Tween20 to block nonspecific binding sites. Incubation with primary antibody was performed overnight at 4°C with 1/75 rabbit anti-cleaved caspase-3 antibody (Cell Signaling Technology, Beverly, MA) in PBS plus 0.05% Tween20. Sections were then incubated for 1 hour with 1/100 biotin-labeled goat anti-rabbit immunoglobulin G (Vector Laboratories, Burlingame, CA) in PBS plus 0.05% Tween20. Subsequently, the sections were incubated for 30 minutes with the ABC kit (Vector Laboratories) and developed for 10 minutes with 3,3′-diaminobenzidine with nickel enhancement. Finally, sections were counterstained with 0.025% toluidine blue in water, dehydrated, and sealed in DePeX mounting medium (BDH Chemicals, VWR International, Poole, England).

Bone Histomorphometry

Histomorphometry was performed mainly on trabecular bone. Static histomorphometry was performed automatically using NIS-Elements AR 2.10 (Nikon GmbH, Düsseldorf, Germany) at 100× magnification. Dynamic histomorphometry was performed semiautomatically using OsteoMeasure (Osteometrics, Atlanta, GA). All measurements were performed according to the American Society of Bone and Mineral Research nomenclature.[20] Mean cortical thickness was assessed manually by measuring both cortices, each at 4 equidistant places. Trabecular bone volume, trabecular bone surface, and trabecular thickness were measured and used to calculate trabecular number and trabecular separation. Besides these structural indices, parameters associated with bone formation were measured, including osteoid volume, osteoid surface, and osteoid thickness. Furthermore, the distance between double tetracycline labels and the labeled (mineralizing) surface was measured under UV light and used to calculate mineral apposition rate, bone formation rate, adjusted apposition rate, and mineralization lag time. Bone resorption was assessed as osteoclast number (measured manually using an integrated eyepiece, Zeiss II; Zeiss, Oberkochen, Germany) and osteoclast surface. All measurements were performed by one investigator.

In addition, the total number of osteocytes, the number of cleaved caspase-3–positive osteocytes, and the number of empty lacunae in the entire trabecular bone area were counted at 200× magnification. Duplicate sections were analyzed by the same investigator. From these data, the following parameters were calculated: total number of osteocytes per bone area, number of empty lacunae per bone area, total number of lacunae per bone area, percentage of positive osteocytes per total number of osteocytes, and percentage of empty lacunae per total number of lacunae.

To compare the data of this CD population with healthy controls, we used healthy control data from 17 men and 26 women (aged 20–60 years),[21] kindly provided by Prof Dr J. E. Compston (Cambridge, England). From this population, sections of 10 age- and sex-matched healthy controls were measured to verify comparability of the histomorphometric indices. The systemic measurement difference between our measurements and those from England was as follows: bone volume, 0.93; trabecular thickness, 0.88; trabecular number, 0.99; osteoid volume, 0.93; and osteoid thickness, 0.98.

Statistical Analysis

Results are expressed as mean ± SD. Histomorphometric indices were compared between patients with CD and healthy controls, as well as between men and women using a 2-tailed Student *t* test for independent samples. Correlations between histomorphometric parameters and biochemical indices were calculated using Pearson's coefficient of correlation. All statistical analyses were performed using SPSS software (version 15.0; SPSS Inc, Chicago, IL). A P value of $<.05$ was considered statistically significant.

Results

Patient Characteristics

Patient characteristics are listed in Table 1. Because several parameters showed relatively high variation in the total population, data are presented for men and women separately. The quiescent state of disease in our CD population was ascertained by a mean CDAI of 93.8 ± 71.7 and a mean C-reactive protein level of 7.3 ± 8.4 mg/L. Disease activity was similar in men and women with CD. Disease duration tended to be longer in male patients with CD ($P = .067$). Levels of biochemical parameters were all within the reference values of our laboratory. However, alkaline phosphatase levels in men with CD were at the upper limit of the reference range and were higher than alkaline phosphatase levels in women with CD (86 vs 60 IU/L; $P = .003$).

Bone Histomorphometry

Histomorphometric data concerning bone structure are summarized in Table 2. Trabecular bone volume and trabecular thickness were reduced in patients with CD when compared with healthy controls ($P < .001$ and $P = .006$, respectively; Figure 1*A* and *B*). Trabecular number was unaffected. Comparison of bone structure parameters between men and women showed no differences within the control population. However, in patients with CD, trabecular bone volume tended to be lower in

Table 1. Patient Characteristics

	Men	Women
Total (n)	11	12
Age (*y*)	44 ± 13	37 ± 8
Body mass index (*kg/m²*)	23 ± 2	23 ± 3
Disease activity		
CDAI (total)	82 ± 61 (0–194)	105 ± 81 (6–322)
C-reactive protein level (*mg/L*)[a]	6.6 ± 6.2 (1–17)	7.9 ± 10.3 (1–38)
Location of disease		
Large intestine/small intestine/both	5/3/3	4/3/5
Bowel resection, yes/no (n)	7/4	6/6
Disease duration (*y*)	19 ± 11	11 ± 8
Age at diagnosis (*y*)	24 ± 11	26 ± 8
Medication use		
Mesalamine derivatives	1	—
Immunosuppressives		
Azathioprine	5	4
6-Mercaptopurine	2	1
Methotrexate	1	1
Biologicals	—	2
No medication	2	4
25-Hydroxyvitamin D_3 (*nmol/L*)[a]	64 ± 19	68 ± 32
Calcium (*mmol/L*)[a]	2.39 ± 0.08	2.31 ± 0.13
Alkaline phosphatase (*U/L*)[a]	86 ± 25[b,c]	60 ± 8
Bone mineral density lumbar spine (*g/cm²*)	0.99 ± 0.15	0.92 ± 0.07
T-score (SD)	−1.22 ± 1.11	−1.37 ± 0.71
Z-score (SD)	−0.79 ± 1.09	−1.17 ± 0.73
Bone mineral density total hip (*g/cm²*)	0.81 ± 0.09	0.81 ± 0.06
T-score (SD)	−1.50 ± 0.64	−1.08 ± 0.47
Z-score (SD)	−1.13 ± 0.62	−0.87 ± 0.56

NOTE. Data represent mean ± SD; in the case of CDAI and C-reactive protein, ranges are shown in parentheses.
[a]Reference ranges obtained from the Department of Clinical Chemistry of the VU University Medical Center: C-reactive protein, <8 mg/L; 25-hydroxyvitamin D_3, 25–150 nmol/L; calcium, 2.20–2.60 mmol/L; alkaline phosphatase, <120 IU/L.
[b]N = 10; alkaline phosphatase value of one patient with a liver function test abnormality was excluded.
[c]Significantly different from women with CD ($P < .01$).

men compared with women ($P = .056$). Trabecular number was lower in men than in women with CD ($P = .037$), and trabecular separation was higher in men ($P = .049$). In Table 3, data of male and female patients with CD are summarized, including variables that were only available in patients with CD.

Histomorphometric data concerning bone remodeling are shown in Table 2. Mineral apposition rate was lower in patients with CD when compared with healthy controls ($P = .007$; Figure 1*E*). None of the patients had signs of osteomalacia. Osteoid volume, mineralizing surface, and bone formation rate were comparable between healthy controls and patients with CD (Figure 1*C*, *D*, and *F*). However, analysis of the individual data revealed a cluster of 9 patients with CD who had a very low mineralizing surface (3.78 ± 1.80) as well as bone formation

Table 2. Bone Structure and Remodeling Parameters in Healthy Controls and Patients With CD

	Abbreviation (unit)	Controls (n = 43)	Patients with CD (n = 23)
Bone volume	BV/TV (*%*)	25.49 ± 4.92	18.90 ± 5.28[a]
Trabecular thickness	Tb.Th (*μm*)	151.42 ± 63.46	120.61 ± 22.16[b]
Trabecular number	Tb.N (*/mm*)	1.86 ± 0.54	1.81 ± 0.42
Osteoid volume	OV/BV (*%*)	2.83 ± 1.47	4.09 ± 3.12
Osteoid thickness	O.Th (*μm*)	7.10 ± 2.56	8.36 ± 2.07
Mineralizing surface	MS/BS (*%*)	10.27 ± 5.20	11.55 ± 7.84
Mineral apposition rate	MAR (*μm/day*)	0.746 ± 0.105	0.671 ± 0.098[b]
Bone formation rate	BFR/BS (*μm³/μm²/day*)	0.080 ± 0.039	0.081 ± 0.059
Adjusted apposition rate	Aj.Ar (*μm/day*)	0.462 ± 0.318	0.534 ± 0.304
Mineralization lag time	Mlt (*day*)	21.25 ± 12.47	17.24 ± 9.39

NOTE. Data represent mean ± SD.
[a]Significantly different from the control group ($P < .001$).
[b]Significantly different from the control group ($P < .01$).

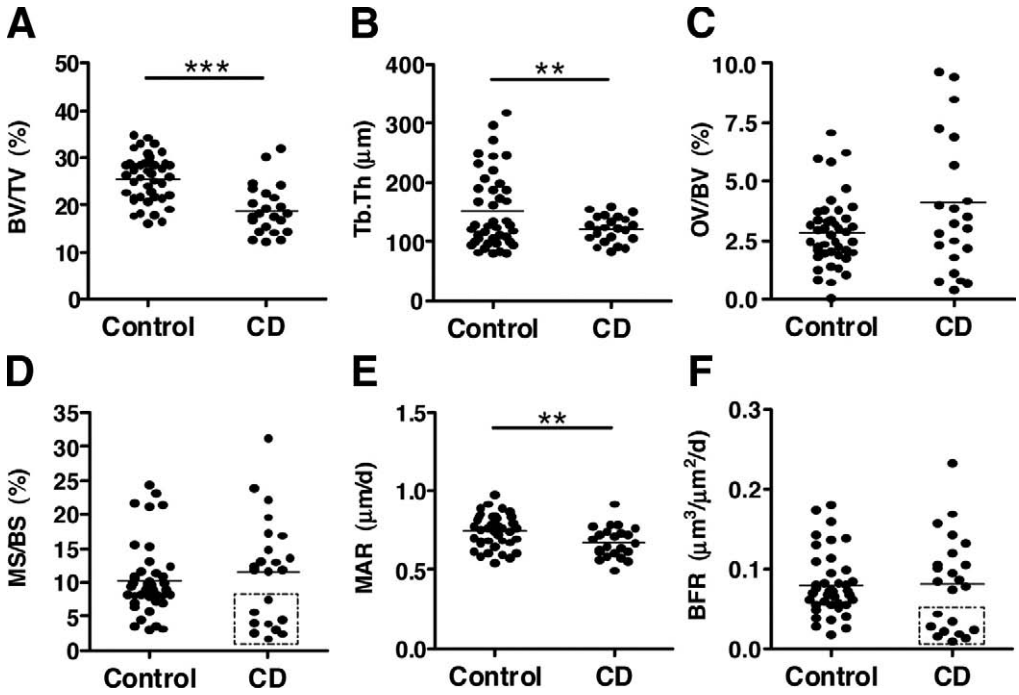

Figure 1. Histomorphometric measurements on bone structure and remodeling in healthy controls and patients with CD. (*A*) Bone volume (BV/TV) is decreased in patients with CD. (*B*) Trabecular thickness (Tb.Th) is decreased in patients with CD. (*C*) Osteoid volume (OV/BV) is unaffected in patients with CD. (*D*) Mineralizing surface (MS/BS) is unaffected in patients with CD; however, a subset of 9 patients with CD had a very low mineralizing surface. (*E*) Mineral apposition rate (MAR) is decreased in patients with CD. (*F*) Bone formation rate (BFR) is unaffected in patients with CD; however, a subset of 9 patients with CD had a very low bone formation rate.

rate (0.022 ± 0.011) in comparison with both the total CD population and healthy controls. Moreover, the 9 patients in this subgroup (4 men and 5 women; age, 43 ± 13 years) turned out to have a low bone volume (15.47 ± 2.90), which was accompanied by a low total hip bone mineral density (T-score, −1.38 ± 0.55). Trabecular thickness and trabecular number were low as well (104.61 ± 16.05 and 1.68 ± 0.33, respectively). Furthermore, these patients had a low osteoid volume (0.42 ± 0.34), a low number of osteoclasts and osteoclast surface (0.19 ± 0.22 and 1.36 ± 0.80, respectively), a low mineral apposition rate (0.59 ± 0.08), and a low adjusted apposition rate (0.29 ± 0.16). Comparison of bone remodeling parameters between men and women showed no statistically significant differences in both healthy controls and patients with CD. Examination of the relationship between

Table 3. Bone Structure and Remodeling Parameters in Male and Female Patients With CD

	Abbreviation (unit)	Men (n = 11)	Women (n = 12)
Cortical thickness	Ct.Th (*μm*)	1060.6 ± 449.5	999.3 ± 418.0
Bone volume	BV/TV (*%*)	16.79 ± 4.58[a]	20.90 ± 5.29
Bone surface	BS/TV (*%*)	3.00 ± 0.46	3.40 ± 0.56
Trabecular thickness	Tb.Th (*μm*)	118.02 ± 24.31	122.81 ± 20.64
Trabecular number	Tb.N (*/mm*)	1.63 ± 0.30[b]	1.98 ± 0.44
Trabecular separation	Tb.Sp (*μm*)	522.9 ± 116.6[b]	417.1 ± 125.2
Osteoid volume	OV/BV (*%*)	4.19 ± 3.05	4.01 ± 3.32
Osteoid surface	OS/BS (*%*)	15.76 ± 6.80	14.29 ± 10.16
Osteoid thickness	O.Th (*μm*)	8.19 ± 1.30	7.82 ± 2.07
Osteoclast number	N.Oc/TA (*/mm²*)	0.46 ± 0.37	0.39 ± 0.25
Osteoclast surface	Oc.S/BS (*%*)	0.87 ± 0.86	0.61 ± 0.33
Mineralizing surface	MS/BS (*%*)	13.30 ± 9.49	9.95 ± 5.95
Mineral apposition rate	MAR (*μm/day*)	0.668 ± 0.097	0.675 ± 0.102
Bone formation rate	BFR/BS (*μm³/μm²/day*)	0.094 ± 0.072	0.070 ± 0.045
Adjusted apposition rate	Aj.Ar (*μm/day*)	0.525 ± 0.285	0.543 ± 0.334
Mineralization lag time	Mlt (*day*)	17.66 ± 10.15	16.86 ± 9.09

NOTE. Data represent mean ± SD.
[a]Tendency towards significantly different from women with CD ($P = .056$).
[b]Significantly different from women with CD ($P < .05$).

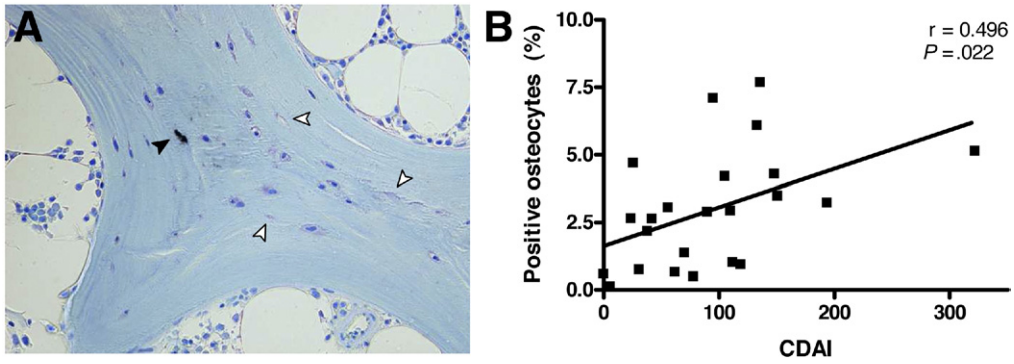

Figure 2. Osteocyte apoptosis in patients with CD. (*A*) Immunohistochemical staining of a bone biopsy section for cleaved caspase-3. The *black arrowhead* indicates an apoptotic osteocyte, and *white arrowheads* indicate empty lacunae. (*B*) Positive correlation between disease activity (CDAI) and the percentage of apoptotic osteocytes.

any of the histomorphometric parameters and disease activity did not reveal statistically significant correlations. Also, no correlations were found between other biochemical parameters and any of the histomorphometric indices.

Osteocyte Apoptosis

Staining for cleaved caspase-3 clearly visualized apoptotic osteocytes, as depicted in Figure 2*A*. In this figure, empty lacunae can be identified as well. In transiliac bone biopsy specimens of patients with CD, the total lacunar density was 293 ± 87 per mm^2 bone consisting of 265 ± 76 osteocytes per mm^2 bone and 28 ± 11 empty lacunae per mm^2 bone. The percentage of empty lacunae over the total number of lacunae was 9.27% ± 3.43%. The number of positive osteocytes over the total number of osteocytes was 3.01% ± 2.23%. None of the parameters differed were statistically significant between men and women with CD.

Examination of the relationship between bone remodeling parameters and osteocyte density as well as osteocyte apoptosis did not reveal statistically significant correlations. Examination of the relationship between disease activity and osteocyte density as well as osteocyte apoptosis showed a positive correlation of CDAI with the percentage of caspase-3–positive osteocytes (r = 0.496, P = .022; see Figure 2*B*).

Discussion

In this study, bone structure and remodeling in transiliac bone biopsy specimens from patients with quiescent CD were investigated. In short, our results indicate that trabecular bone volume was lower in patients with CD than in healthy age- and sex-matched controls. The reduction of bone mass was more pronounced in men than in women and was characterized by a reduction in trabecular thickness as well as trabecular number. Besides structural differences, we observed a change in bone remodeling as indicated by a reduced mineral apposition rate in this CD population when compared with healthy controls. Bone remodeling parameters did not differ between sexes.

The observed low bone mass in patients with quiescent CD is in agreement with previous studies on bone histomorphometric analysis in patients with IBD.[12,14] We showed that the mild bone mass deficit in patients with quiescent CD is related to thinning of trabeculae. Only one other study published details on bone microarchitecture in patients with IBD, reporting a mild cortical bone deficit but a normal amount and structure of trabecular bone in children with newly diagnosed IBD.[13] In our study, cortical thickness in bone of patients with CD was comparable to values in healthy controls (J. Compston, personal communication). This discrepancy might be due to a different response of the developing skeleton to IBD than the mature skeleton. In addition, patients in the study of Ward et al were included at diagnosis, before treatment had been instituted, which might be a cause of the difference as well.

The relatively small reduction in bone formation we observed in patients with quiescent CD was also reported by Croucher et al.[12] It must be mentioned that, in contrast to our population, the majority of their patients received prednisolone therapy at time of the bone biopsy. This is likely to have been a contributory factor to the bone loss and might explain the stronger reduction in bone formation they observed in comparison with our findings.

Interestingly, in our study population, a subset of patients had histomorphometric characteristics resembling osteoporosis rather than osteopenia. In these patients, all bone formation indices were low and comparable to the extent of reduction of bone formation seen by Croucher et al. Because recent corticosteroid treatment was an exclusion criterion in this study, current corticosteroid therapy cannot be the cause of the reduced bone formation in our patients. Sex, age, current medication use, and number of patients with small bowel resection(s) in the past were similar in the subset and total population. Unfortunately, prospective data on life-

time cumulative dose of glucocorticoids and the history of number as well as severity of relapses are not available in the current study. Both aspects could well be related to a more severely affected bone phenotype. However, a retrospective estimate showed no statistically significant correlation between cumulative glucocorticoid dose and any of the bone formation parameters.

Glucocorticoids inhibit differentiation and activity of osteoblasts and its precursor cells and, moreover, induce osteoblast apoptosis.[22] An advantage of this study is that only patients without recent corticosteroid treatment were included. Inherent to this aspect, patients in this study were in a quiescent state of disease, which is a poorly investigated but interesting group of patients because there are indications that the cumulative effects of even small excesses in cytokine levels over many months can be clinically relevant. One of our findings supporting this theory is that bone of male patients with CD, who tended toward longer disease duration, was more severely affected than bone of female patients. Men with CD in this cohort had lower bone volume and both less and thinner trabeculae than women with CD. The latter aspect is in contrast to observations in healthy controls describing no difference in bone structure between men and women.[21] Moreover, in postmenopausal populations, bone loss due to the loss of whole trabeculae has been reported mainly in women.[23] A higher bone turnover in male patients with CD in this cohort, as indicated by higher levels of alkaline phosphatase than in female patients, might partially explain this finding. However, direct differences in bone remodeling indices could not be detected between men and women with CD, which suggests the existence of differences between male and female patients in the past.

Bone resorption data were not available in the healthy control population, but when compared with the literature, osteoclast number and osteoclast surface seemed to be low in our patients with CD.[16,24] Our data are in concordance with previous studies addressing bone resorption in patients with IBD, which showed a slight decrease in bone resorption indices in both children and adults with IBD.[12,13] These findings raise the question whether treatment with antiresorptive agents is beneficial to all patients with CD with osteoporosis or osteopenia. However, additional data on eroded surface, resorption cavities, and serum levels of bone resorption markers are necessary to make firm conclusions on bone resorption in this study population.

Osteocyte apoptosis interrupts signaling between osteocytes and the effector cells of bone: osteoblasts and osteoclasts. Therefore, a lack of osteocytes or inefficacy of their function can be related to a defective bone structure and remodeling. As far as we know, osteocyte density and apoptosis in transiliac bone biopsy specimens from patients with CD have not been studied before. From comparison of our findings with the literature, it appears that osteocyte density in bone of patients with CD is within the range observed in healthy controls.[15,16] Literature on osteocyte apoptosis in healthy controls is limited, because the assessment of apoptotic osteocytes in bone specimens is relatively new in bone morphometry. Nonetheless, the percentage of apoptotic osteocytes in our CD population is comparable to the percentage observed in hip fracture controls by Sambrook et al.[25] However, the percentage of empty lacunae is relatively high when compared with the controls in that study and resembles more the percentage of empty lacunae observed in their prednisone-treated patients with rheumatoid arthritis. This finding may be explained by an increased osteocyte apoptosis in the past, because empty lacunae mark sites where osteocytes have died previously. This hypothesis is corroborated by the positive correlation we observed between disease activity (CDAI) and the percentage of apoptotic osteocytes. In that case, osteocyte apoptosis might have been increased during an active period of disease in patients with CD. However, C-reactive protein level did not correlate with the percentage osteocyte apoptosis. Because CDAI is inaccurate in the low range, the correlation of CDAI with osteocyte apoptosis might be due to chance. Therefore, the biological relevance of this finding remains to be elucidated.

A drawback of bone histomorphometry is a considerable measurement variation. Differences in staining techniques, methods to measure the specimen, as well as intersection and interobserver variance can be substantial. The number of patients studied is relatively small, but it still is the largest series in well-characterized patients with IBD. Another limitation is that patients with IBD reflect a heterogeneous population. In this study, only patients with CD were included. In addition, these patients were in a quiescent state of disease and had not used glucocorticoids for at least 3 months before inclusion. These restrictions to patient inclusion should have reduced the impact of heterogeneity in this study. However, we still might not have had enough statistical power to detect certain differences and correlations.

In conclusion, in this study, for the first time a cohort of adult patients with quiescent CD was evaluated using bone histomorphometry. In these patients, bone mass is reduced as characterized by trabecular thinning. Furthermore, our results show that CD-associated bone loss is caused by a reduced bone formation at the tissue level, possibly as a consequence of decreased osteocyte viability in the past.

References

1. Bernstein CN, Leslie WD. Review article: osteoporosis and inflammatory bowel disease. Aliment Pharmacol Ther 2004;19:941–952.
2. Vestergaard P. Bone loss associated with gastrointestinal disease: prevalence and pathogenesis. Eur J Gastroenterol Hepatol 2003;15:851–856.

3. Bernstein CN, Leslie WD. The pathophysiology of bone disease in gastrointestinal disease. Eur J Gastroenterol Hepatol 2003;15: 857–864.
4. Moschen AR, Kaser A, Enrich B, et al. The RANKL/OPG system is activated in inflammatory bowel disease and relates to the state of bone loss. Gut 2005;54:479–487.
5. Robinson RJ, Iqbal SJ, Abrams K, et al. Increased bone resorption in patients with Crohn's disease. Aliment Pharmacol Ther 1998; 12:699–705.
6. Dresner-Pollak R, Karmeli F, Eliakim R, et al. Increased urinary N-telopeptide cross-linked type 1 collagen predicts bone loss in patients with inflammatory bowel disease. Am J Gastroenterol 2000;95:699–704.
7. Gilman J, Shanahan F, Cashman KD. Altered levels of biochemical indices of bone turnover and bone-related vitamins in patients with Crohn's disease and ulcerative colitis. Aliment Pharmacol Ther 2006;23:1007–1016.
8. Abitbol V, Roux C, Chaussade S, et al. Metabolic bone assessment in patients with inflammatory bowel disease. Gastroenterology 1995;108:417–422.
9. Bischoff SC, Herrmann A, Goke M, et al. Altered bone metabolism in inflammatory bowel disease. Am J Gastroenterol 1997;92: 1157–1163.
10. Bjarnason I, Macpherson A, Mackintosh C, et al. Reduced bone density in patients with inflammatory bowel disease. Gut 1997; 40:228–233.
11. E.F.Eriksen BLMK. The cellular basis of osteoporosis. Spine: state of the art reviews. 8th ed. 1994.
12. Croucher PI, Vedi S, Motley RJ, et al. Reduced bone formation in patients with osteoporosis associated with inflammatory bowel disease. Osteoporos Int 1993;3:236–241.
13. Ward LM, Rauch F, Matzinger MA, et al. Iliac bone histomorphometry in children with newly diagnosed inflammatory bowel disease. Osteoporos Int 2010;21:331–337.
14. Hessov I, Mosekilde L, Melsen F, et al. Osteopenia with normal vitamin D metabolites after small-bowel resection for Crohn's disease. Scand J Gastroenterol 1984;19:691–696.
15. Qiu S, Rao DS, Palnitkar S, et al. Age and distance from the surface but not menopause reduce osteocyte density in human cancellous bone. Bone 2002;31:313–318.
16. Mullender MG, Tan SD, Vico L, et al. Differences in osteocyte density and bone histomorphometry between men and women and between healthy and osteoporotic subjects. Calcif Tissue Int 2005;77:291–296.
17. Qiu S, Rao DS, Palnitkar S, et al. Reduced iliac cancellous osteocyte density in patients with osteoporotic vertebral fracture. J Bone Miner Res 2003;18:1657–1663.
18. van Essen HW, Holzmann PJ, Blankenstein MA, et al. Effect of raloxifene treatment on osteocyte apoptosis in postmenopausal women. Calcif Tissue Int 2007;81:183–190.
19. Lennard-Jones JE. Classification of inflammatory bowel disease. Scand J Gastroenterol Suppl 1989;170:2–6.
20. Parfitt AM, Drezner MK, Glorieux FH, et al. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 1987;2:595–610.
21. Vedi S, Compston JE, Webb A, et al. Histomorphometric analysis of bone biopsies from the iliac crest of normal British subjects. Metab Bone Dis Relat Res 1982;4:231–236.
22. Dalle CL, Bertoldo F, Valenti MT, et al. Histomorphometric analysis of glucocorticoid-induced osteoporosis. Micron 2005;36: 645–652.
23. Kimmel DB, Recker RR, Gallagher JC, et al. A comparison of iliac bone histomorphometric data in post-menopausal osteoporotic and normal subjects. Bone Miner 1990;11:217–235.
24. Ott SM, Oleksik A, Lu Y, et al. Bone histomorphometric and biochemical marker results of a 2-year placebo-controlled trial of raloxifene in postmenopausal women. J Bone Miner Res 2002; 17:341–348.
25. Sambrook PN, Hughes DR, Nelson AE, et al. Osteocyte viability with glucocorticoid treatment: relation to histomorphometry. Ann Rheum Dis 2003;62:1215–1217.

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Conflicts of interest

The authors disclose no conflicts.

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