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Original Article

Reduced Bone Formation in Patients with Osteoporosis Associated with Inflammatory Bowel Disease

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Abstract. The pathophysiology of bone loss associated with inflammatory bowel disease has not been clearly defined. In this study we have performed a detailed histomorphometric analysis of iliac crest bone obtained from 19 patients with inflammatory bowel disease in whom a diagnosis of osteoporosis had been made. Eleven subjects were receiving prednisolone at the time of their biopsy. Comparison with control values demonstrated a highly significant reduction in trabecular bone area in the patient group ($p<0.001$). Wall width, adjusted appositional rate and bone formation rate were all significantly reduced in the patient group ($p<0.001$) and the formation period was significantly increased ($p<0.001$). Resorption cavities were slightly smaller in the patient group, differences in maximum cavity depth and cavity length achieving statistical significance ($p<0.005$ and $p<0.05$ respectively). The mineral appositional rate was significantly reduced in the patients with inflammatory bowel disease ($p<0.001$) and the mineralization lag time significantly increased ($p<0.001$); however, osteoid area, perimeter and seam width were not significantly different from controls. These results demonstrate that osteoporosis associated with inflammatory bowel disease is characterized by reduced bone formation at the cellular and tissue level; the proportionately greater change in wall width than in resorption cavity depth is consistent with a negative remodelling balance. Although none of the patients had osteomalacia as defined by the criteria of increased osteoid seam width and mineralization lag time, the higher mineralization lag time in the patient group indicates a mild mineralization defect.

Keywords: Bone formation; Bone histomorphometry; Inflammatory bowel disease; Osteoporosis

Introduction

Inflammatory bowel disease is associated with an increased prevalence of osteoporosis [1–5] and increased rates of spinal bone loss have been demonstrated in some patients [6]. The pathogenesis of bone loss is not fully understood but it is likely to be multifactorial; steroid therapy, sex hormone deficiency, malnutrition and calcium and vitamin D deficiency are all possible contributory factors. The clinical significance of the observed bone loss is emphasized by the development of multiple vertebral fractures, height loss and persistent pain in some relatively young patients [5].

Very little is known about the pathophysiology of osteoporosis associated with inflammatory bowel disease. Hessov et al. [7] demonstrated reduced iliac crest trabecular bone mass in unselected outpatients with Crohn's disease and small bowel resection; since bone turnover was normal, they hypothesized that the reduction in bone mass must be due to remodelling imbalance, although wall width and resorption depth were not measured. In the present study we have assessed both bone turnover and remodelling balance in a group of patients with inflammatory bowel disease in whom a diagnosis of osteoporosis had been made on the basis of low bone mass and/or the presence of vertebral fractures.

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Subjects and Methods

Patients and Controls

Trans-iliac crest bone biopsies were obtained after informed consent from 19 of 23 patients with inflammatory bowel disease in whom osteoporosis was diagnosed in a previous study [5]. All had reduced bone mineral density in the spine and/or radius greater than 2 standard deviations below the normal mean value and in 6 patients one or more vertebral fractures were present. The group comprised 11 women and 8 men, aged 21–77 years (mean 42 years); of these 16 had Crohn's disease (1 large bowel only, 12 small bowel only; 3 large and small bowel), 2 had ulcerative colitis and 1 had carcinoid. The duration of diagnosed disease ranged from 3 to 28 years (mean 13 years). All but 3 patients had undergone one or more bowel resection. The cumulative lifetime dose of prednisolone at the time of the biopsy ranged from 0 to 49 g (median 15 g); 11 patients were receiving prednisolone therapy at the time of the bone biopsy. Three premenopausal patients were receiving 1α-hydroxyvitamin D_3, and 1 was receiving calcium and vitamin D tablets. Three premenopausal patients aged 34, 38 and 42 years were amenorrhoeic. None had received hormone replacement therapy at the time of the biopsy.

Control values were obtained from a group of 57 healthy subjects, comprising 24 men and 33 women aged 19–80 years (mean 46 years) who gave informed consent to undergo bone biopsy during general anaesthesia for a minor surgical procedure. Details of these subjects have been described previously [8,9]. Of the original 57 subjects a subset of 41, comprising 20 men and 21 women aged 22–80 years (mean 51 years), provided the control group for comparison of resorption cavity characteristics.

Bone Histomorphometry

Specimens were fixed in 10% phosphate-buffered formalin and embedded in methylmethacylate (British Drug House Chemicals, Dorset) (control group) or LR White embedding medium (London Resin Company, Surrey) (patient group). Eight micrometre undecalcified sections were cut with a Jung K microtome and stained by the von Kossa technique using a van Gieson counterstain, solochrome cyanin R, or 1% toluidine blue (pH 4.2). Undecalcified sections 14 μm thick were mounted unstained for fluorescence microscopy.

Measurement of cancellous bone area (B.Ar/T.Ar), osteoid area (O.Ar/T.Ar) and osteoid perimeter (O.Pm/B.Pm) were made on von Kossa-stained sections, using a Zeiss integrating micrometer-disk turret 1 containing 25 points and 7 parallel lines. Osteoid width (O.Wi) was measured directly on von Kossa-stained sections using an eyepiece micrometer (Graticles, Tonbridge, Kent). The mean of 4, or occasionally 8 equidistant measurements, from at least 20 osteoid-covered perimeters, was taken as the mean value for the biopsy. Wall width (W.Wi) was measured, using an eyepiece micrometer, at × 100 magnification, as the distance between the cement line and mineralized bone surface without evidence of bone resorption or osteoid seams. Measurements were made under polarized light using sections stained with solochrome cyanin R (control) or toluidine blue (patient group). Four approximately equidistant measurements were made per completed bone packet, keeping the micrometer perpendicular to the cement line. A minimum of 30 packets was measured for each biopsy. Comparison of wall width values obtained in 5 biopsies from control subjects demonstrated no significant difference between sections stained with solochrome cyanin (mean (SD) 49.9 (6.4) μm) and those stained with toluidine blue (47.8 (4.2) μm; p = NS). All values are expressed in two dimensions.

Tetracycline labelling was assessed using an interactive computerized technique. The LED from a digitizer tablet was projected on to the microscope field of view using a drawing arm attachment. The bone perimeter (B.Pm), single- and double-labelled perimeters were traced using the digitizer tablet. Mineralizing perimeter (M.Pm/B.Pm) was calculated as double-labelled perimeter +0.5 single-labelled perimeter. An average of 40 fields was measured from two or more unstained sections, at a magnification of × 156 using fluorescence microscopy. The distance between double-labelled perimeters (L.Wi) was measured as the mean of 4 approximately equidistant points from two or more sections.

The following indices were calculated from primary measurements [10]:

Mineral appositional rate (MAR, μm/day)	= L.Wi/12
Adjusted appositional rate (Aj.AR, μm/day)	= MAR * M.Pm/O.Pm)
Bone formation rate (BFR/B.Pm, $\mu m^2/\mu m/day$)	= MAR * (M.Pm/B.Pm)
Formation period (FP, days)	= W.Wi/Aj.AR
Active formation period (FP(A+), days)	= W.Wi/MAR
Osteoid maturation period (Omt, days)	= O.Wi/MAR
Mineralization lag time (Mlt, days)	= O.Wi/Aj.AR
Activation frequency (Ac.F, $year^{-1}$)	= BFR/B.Pm/W.Wi

Measurement of Resorption Cavity Characteristics

Details of this method have been described previously [11]. Having identified a resorption cavity the image is stored in the memory of an IBAS II image analyser (Kontron, FRG) and displayed on a television monitor. The two end points of the resorption cavity are identified using a screen cursor and entered into the image analyser. The positions of the intersections between the circles and the original bone surface on each side of the cavity are also entered. A smooth continuous curve, known as a cubic spline, is drawn through the defined points. To maintain the smooth continuity of the curve

and bone surfaces on each side of the cavity, additional points at maximum deviation are added to refine the curve fit. This line is used as a baseline for the assessment of cavity characteristics.

The following indices were obtained by direct measurement or calculation:

Cavity count/B.Pm (/mm)	Number of cavities per millimetre of cancellous bone perimeter
Cavity count/T.Ar (/mm^2)	Number of cavities per square millimetre of medullary area
Maximum depth (μm)	Maximum cavity depth
Mean depth (μm)	Mean cavity depth (from approximately 4 equidistant points)
Reconstructed length (μm)	Length of resorption cavity (measured along the cubic spline between the 2 resorption end points)
Eroded length (μm)	Length of resorption cavity measured along the base of the cavity
Cavity area (μm^2)	Area of resorption cavity
Reconstructed perimeter/B.Pm (%)	Percentage of cancellous perimeter occupied by resorption cavities (reconstructed length)
Eroded perimeter/B.Pm (%)	Percentage of cancellous perimeter occupied by resorption cavities (eroded ' 'gth)
Bone remodelled/B.Ar (%)	..rcentage bone remodelled calculated as the cavity area divided by the cavity area plus the bone area and multiplied by 100

Resorption cavities were identified at a magnification of × 375 or occasionally × 750 under polarized light. All crenated perimeters with lamellae the end points of which terminated at the bone/marrow interface were included. No attempt was made to measure cavities that occurred at the end of trabeculae and had resulted in trabecular penetration. A minimum of 20 cavities was assessed from between 2 and 13 sections in the patients with inflammatory bowel disease, the mean number ±SD of cavities in each biopsy being 21.8 ± 2.1. In 6 biopsies assessment of cavity characteristics was not possible due to insufficient numbers of sections. Between 2 and 13 sections were examined in the control subjects, the mean ±SD of cavities examined in each biopsy being 25.2 ± 2.3.

Statistics

Log transformation was performed to normalize non-normally distributed data. Differences between variables in control subjects and those with osteoporosis secondary to inflammatory bowel disease were tested by a two-tailed unpaired *t*-test. The relationships between disease duration, cumulative prednisolone dose and bone histomorphometric indices were examined by Pearson's correlation. Data are expressed as geometric means (anti-log of the mean of the log values) and 95% ranges.

Results

Values for the standard histomorphometric variables from patients with inflammatory bowel disease and control subjects are shown in Table 1. Cancellous bone area was significantly lower in the patient group ($p<0.001$) than in the control group. Values for osteoid area, perimeter and width did not differ significantly between the patient and control groups and all values in the patient group were within 2 standard deviations of the normal mean value. Mineral appositional rate was significantly decreased ($p<0.001$) and osteoid maturation period and mineralization lag time significantly increased ($p<0.01$ and 0.001 respectively) (Fig. 1) in the patient group. Wall width, adjusted appositional rate and bone formation rate at the tissue level were all

Table 1. Histomorphometric variables in patients with inflammatory bowel disease and control subjects

	Control (n = 57)	Inflammatory bowel disease (n = 19)	p value
B.Ar/T.Ar (%)	24.1[a] (15.8–36.9)[b]	13.21 (3.53–49.5)	<0.001
O.Ar/T.Ar (%)	2.85 (0.86–9.48)	3.82 (0.559–26.1)	NS
O.Pm/B.Pm (%)	19.6 (6.27–61.2)	22.02 (6.47–75.0)	NS
O.Wi (μm)	7.08 (3.48–14.44)	7.51 (4.31–13.1)	NS
W.Wi (μm)	51.6 (35.8–74.4)	43.3 (31.1–60.1)	<0.001
M.Pm/B.Pm (%)	9.35 (3.31–26.4)	4.06 (1.25–1.32)	<0.001
MAR (μm/day)	0.731 (0.531–1.007)	0.600 (0.452–0.797)	<0.001
Aj.AR (μm/day)	0.355 (0.088–1.435)	0.105 (0.016–0.702)	<0.001
BFR/B.Pm (μm^2/μm/day)	0.070 (0.023–0.214)	0.024 (0.007–0.083)	<0.001
FP (days)	143.6 (38.4–537.0)	420.7 (70.8–2501.0)	<0.001
FP(A+) (days)	70.1 (47.1–104.3)	72.3 (44.6–117.2)	NS
Ac.F (year)[1]	0.502 (0.158–1.59)	0.205 (0.065–0.644)	<0.001
Omt (days)	9.97 (5.09–19.54)	12.9 (8.21–20.4)	<0.001
Mlt (days)	20.5 (5.10–82.7)	78.8 (25.7–559.6)	<0.001

[a]Geometric mean; [b]95% range.

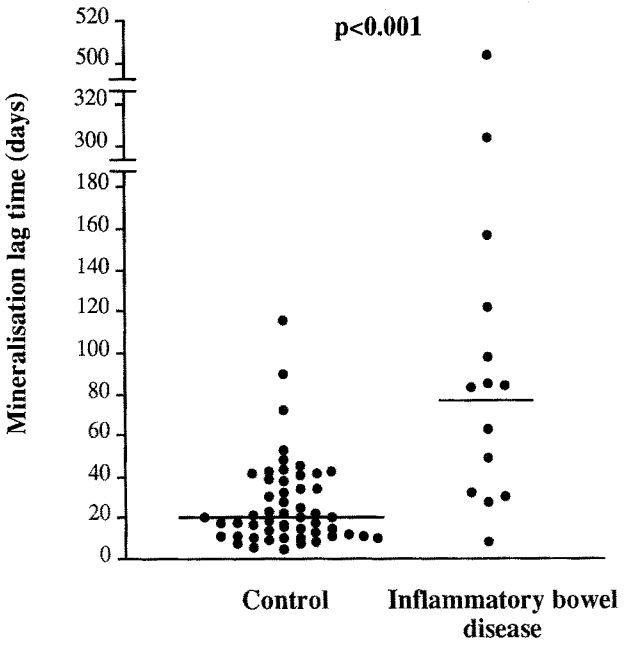

Fig. 1. Mineralization lag time in the patients and control subjects. The geometric mean value is shown by the continuous horizontal line.

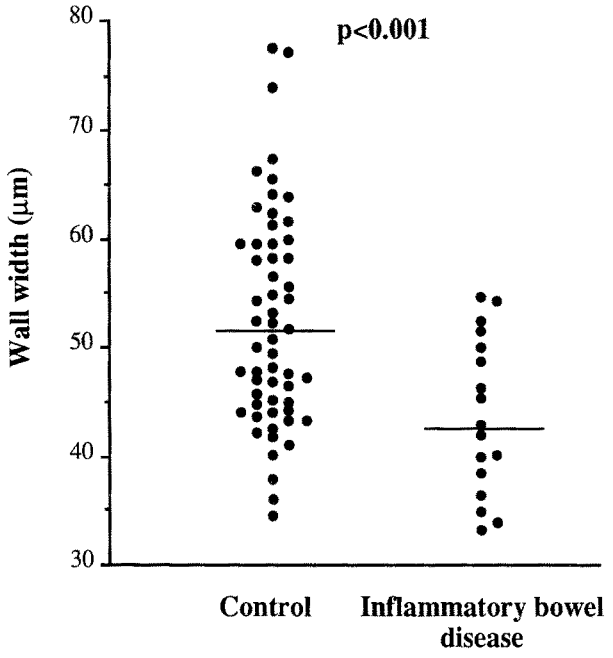

Fig. 2. Wall width in the patients and control subjects. The geometric mean value is shown by the continuous horizontal line.

significantly reduced in the patient group ($p<0.001$) (Figs. 2 and 3). Formation period was significantly increased in patients ($p<0.001$); however, active formation period was not significantly different between patient and control groups. The calculated activation frequency was significantly reduced in the patient group ($p<0.001$).

Values for directly measured and calculated resorption cavity characteristics are shown in Table 2. Maximum cavity depth was significantly decreased in the patients with inflammatory bowel disease when compared with controls ($p<0.005$); the mean cavity depth was also smaller in the patient group although this difference did not achieve statistical significance. Reconstructed and eroded length were significantly lower in the patients than in controls ($p<0.01$ and 0.05 respectively). Cavity area showed a non-significant decrease in the patient group; there were no differences in eroded perimeter or cavity number between the patient and control groups.

Examination of the relationships between disease duration, prednisolone dosage and histomorphometric indices revealed a significant negative correlation between the cumulative dose of prednisolone and mean and maximum cavity depth ($r = -0.613$, $p<0.05$ and $r = -0.661$, $p<0.05$, respectively). No correlations were found between disease duration and any of the bone histomorphometric indices.

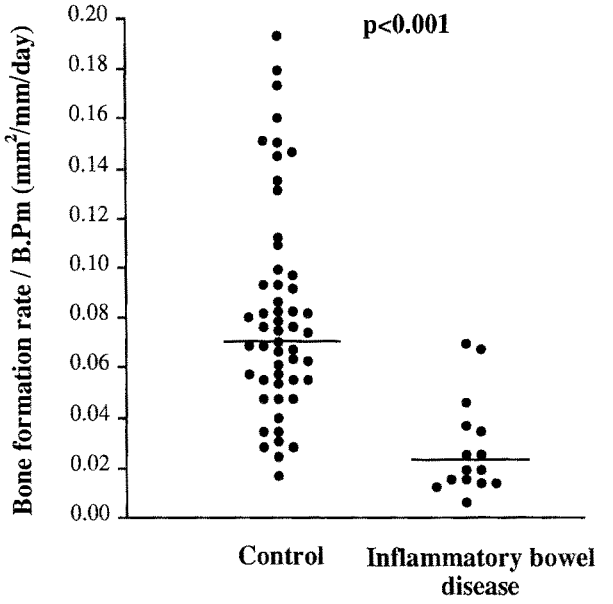

Fig. 3. Bone formation rate in the patients and control subjects. The geometric mean value is shown by the continuous horizontal line.

Discussion

Our results indicate that osteoporosis associated with inflammatory bowel disease is characterized by reduced bone formation at the cellular and tissue level. Cancellous bone area was considerably reduced, emphasizing the severity of osteoporosis in this cohort, although iliac crest bone area does not always reflect accurately bone mass at other skeletal sites [12]. There was also a highly significant reduction in wall width; in contrast, resorption cavities were only slightly smaller than in healthy controls and statistical significance was achieved only for the maximum cavity depth and the length of individual cavities. This suggests that a negative

Table 2 Resorption cavity characteristics in patients with inflammatory bowel disease and control subjects

	Control (n = 41)		Inflammatory bowel disease (n = 13)		p value
Mean cavity depth (μm)	21.1[a]	(14.1–31.4)[b]	18.7	(13.7–25.5)	NS
Maximum cavity depth (μm)	34.2	(22.8–51.3)	27.9	(20.5–38.0)	<0.005
Reconstructed length (μm)	208.5	(145.1–299.6)	178.0	(138.8–228.4)	<0.01
Eroded length (μm)	239.8	(170.2–338.0)	210.2	(167.1–264.4)	<0.05
Cavity area (μm^2)	3467	(1820–6425)	2922	(1780–1798)	NS
Reconstructed perimeter/B.Pm (%)	1.68	(0.653–4.32)	1.46	(0.511–4.15)	NS
Eroded perimeter/B.Pm (%)	1.94	(0.760–4.93)	1.72	(0.596–4.95)	NS
Cavity count/B.Pm (/mm)	0.231	(0.096–0.558)	0.178	(0.078–0.409)	NS
Cavity count/T.Ar (/mm)	0.072	(0.031–0.169)	0.073	(0.031–0.173)	NS
Bone remodelled (%)	0.575	(0.235–1.409)	0.497	(0.124–1.995)	Ns

[a]Geometric mean; [b]95% range.

remodelling imbalance was primarily responsible for bone loss, since low bone turnover *per se* does not affect bone mass although it does result in an increase in mean bone age [13]. However, since our measurement technique includes resorption cavities at all stages of development, remodelling balance cannot be accurately calculated.

Calculation of dynamic indices of bone formation revealed significant reductions in adjusted appositional rate and bone formation rate at the tissue level with a marked increase in the formation period; the active formation period, however, did not differ significantly between the two groups, indicating a normal osteoblastic life span but periodic cessation or slowing down of mineralization in the patient group. There was also a significant reduction in the mineral appositional rate, which is most likely to be secondary to the reduced rate of matrix formation. Osteomalacia has been described in association with malabsorption [14] but the normal osteoid seam width in all patients in the present study excludes this diagnosis, although the increased mineralization lag time indicates a mild mineralization defect; the low osteoblastic appositional rate in the majority of our patients would preclude the development of osteomalacia. This combination of normal osteoid seam width and increased mineralization lag time was also noted by Hessov et al. [7] in some patients with Crohn's disease, although in their study no reduction in the mineral appositional rate was found. Serum 25-hydroxyvitamin D and parathyroid hormone concentrations in our patients have been reported previously [5] and were within the normal range in all but 1 patient who had a low serum 25-hydroxyvitamin D but a normal serum parathyroid hormone concentration; similar findings were reported by Hessov et al. [7]. Other groups have reported reduced serum levels of 25-hydroxyvitamin D in patients with Crohn's disease [15–17]; 4 of our patients were receiving vitamin D supplementation prophylactically but the histomorphometric indices of mineralization in these were similar to those in unsupplemented cases. Evidence for secondary hyperparathyroidism was also lacking in our patients; none showed abnormally elevated values for eroded perimeter and the mineralization perimeter, which reflects activation frequency, was sign ificantly reduced in the patient group as a whole and below the upper limit of normal in all cases. In addition, calculated activation frequency was significantly lower in the patient group, although this derived parameter may not always reflect true activation frequency [13].

Assessment of resorption indicated that resorption cavity size was slightly reduced in the patient group and that the number of cavities, whether expressed in terms of bone perimeter or total area, was similar to that found in the control group. In situations where bone turnover is reduced, a reduced number of cavities would be expected unless uncoupling of the normal remodelling sequence resulted in failure of bone formation at sites of resorption. This phenomenon has been reported in patients with chronic liver disease [18,19], in whom low bone turnover is associated with a normal or even increased eroded surface and presumably reflects an increase in the number of cavities in which the remodelling process is aborted during or at the end of the resorptive phase.

The patients included in our study were heterogeneous in many respects, including age, sex, disease activity and nutrition, severity of osteoporosis, degree of malabsorption, drug therapy and sex hormone status. This heterogeneity is inevitable in a disease state which varies with respect to severity and extent and which undergoes relapse and remission; factors such as corticosteroid therapy and sex hormone deficiency are an integral part of the complex clinical profile of inflammatory bowel disease, the fluctuating course of which is likely to be reflected by varying perturbations in bone remodelling which cannot be detected in a single biopsy. The majority of patients had received corticosteroid therapy and this is likely to have been a contributory factor to bone loss [20]; the failure to demonstrate any relationship between lifetime cumulative dose and histomorphometric indices of bone formation may reflect both the natural history of inflammatory bowel disease and the difficulty involved in retrospective calculation of lifetime corticosteroid dosage, although significant negative correlations were observed between lifetime

prednisolone dose and mean and maximum resorption cavity depth. Similarly, sex hormone deficiency and/or episodes of secondary hyperparathyroidism during active disease or associated with vitamin D deficiency during the winter months [21] may have contributed to bone loss in the past even though no evidence for increased bone turnover could be demonstrated in the present study. The severity of osteoporosis in some of our patients indicates either that their bone mass was low at skeletal maturity and/or that increased bone turnover occurred in the past, since a negative remodelling balance in the presence of low bone turnover will result in only slow bone loss; the latter explanation would be consistent with our demonstration that some patients with inflammatory bowel disease exhibit greatly increased rates of bone loss [6].

Although the pathogenesis and evolution of osteoporosis associated with inflammatory bowel disease remain poorly defined, this study clearly demonstrates that established bone disease is characterized by low bone turnover and negative remodelling balance. The clinical significance of this bone disease requires further study, but our observation of multiple vertebral fractures in some relatively young patients [15] emphasizes the need for preventive and therapeutic measures. Hormone replacement therapy should be given to perimenopausal women and to those with long-standing amenorrhoea, and vitamin D and calcium supplements should be given to those patients at risk from privational vitamin D deficiency or with low dietary calcium intake. Corticosteroid therapy should be avoided whenever possible and, where necessary, should be constantly reviewed and kept to a minimum. The treatment of established osteoporosis with regimes currently used in postmenopausal osteoporosis has not been evaluated and constitutes an important area for future research.

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