

# MARKERS OF BONE METABOLISM IN POSTMENOPAUSAL WOMEN WITH RHEUMATOID ARTHRITIS

## Effects of Corticosteroids and Hormone Replacement Therapy

G. M. HALL, T. D. SPECTOR, and P. D. DELMAS

**Objective.** To investigate bone metabolism in postmenopausal women with rheumatoid arthritis (RA) treated with or not treated with corticosteroids, and the response to hormone replacement therapy (HRT).

**Methods.** One hundred six RA patients were divided into those taking low-dose steroids (RA+; n = 35) and those not (RA-; n = 71) and randomly allocated to receive HRT or calcium for 2 years. Bone formation markers included serum osteocalcin (OC) and bone-specific alkaline phosphatase, and resorption markers included urinary deoxypyridinoline (DPyr) and CrossLaps (XL). Bone mineral density (BMD) was measured annually using dual x-ray absorptiometry.

**Results.** OC levels were significantly lower in both the RA+ and RA- groups compared with 112 healthy control subjects ( $P < 0.01$  and  $P < 0.05$ , respectively), but were similar in the 2 RA groups. DPyr and XL levels were elevated in the RA+ group compared with the RA- group ( $P < 0.05$ ) but were similar between the RA- group and controls. OC was negatively correlated with parameters of disease activity ( $P < 0.05$ ). After HRT, XL excretion decreased significantly in the overall RA group. Three-month changes in XL correlated with 2-year changes in spinal BMD ( $P < 0.01$ ).

**Conclusion.** Bone metabolism may be uncoupled in chronic RA. Bone formation appears to be reduced, partly reflecting disease activity, whereas resorption is

increased only in steroid users. HRT reduces resorption in RA irrespective of steroid usage, emphasizing its value in the treatment of postmenopausal women with RA.

Patients with rheumatoid arthritis (RA) have an increased likelihood of developing fractures (1), partly due to reduced bone mass (2,3). The mechanisms accounting for osteopenia in RA are unclear, but biochemical markers of bone formation and resorption offer an insight into the effects of RA and its treatment on bone metabolism. Some biochemical studies have demonstrated increased, and others reduced, bone turnover in RA, though their techniques and study populations have varied (4-6). Corticosteroids may negatively affect both bone formation and resorption (7), and we have found that hormone replacement therapy (HRT), an antiresorptive treatment, increased spinal bone mass in patients receiving steroids (8). Other cross-sectional studies also suggest a favorable effect of HRT on bone mass in RA (9,10).

In the present investigation, we analyzed markers of bone metabolism in patients with RA, some receiving steroids, who participated in a 2-year randomized controlled study of HRT. We assessed (a) the effect of disease activity and low-dose steroid treatment on baseline markers, (b) subsequent changes in markers with HRT, and (c) the correlation between short-term changes in markers and long-term changes in bone mass.

### PATIENTS AND METHODS

**Patients.** One hundred six postmenopausal women (age 45-65 years) with definite RA according to the 1958 criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (11) were recruited from 5 rheumatology outpatient centers. Sixty percent of the patients were receiving a slow-acting antirheumatic drug

Supported in part by a grant from Ciba Pharmaceuticals, Horsham, UK.

G. M. Hall, MRCP, T. D. Spector, MD, MRCP: St. Thomas' Hospital, London, UK; P. D. Delmas, MD: INSERM Unit 403, Hôpital Edouard Herriot, Lyon, France.

Address reprint requests to T. D. Spector, Department of Rheumatology, St. Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK.

Submitted for publication July 13, 1994; accepted in revised form January 4, 1995.

(SAARD) and 33% were receiving corticosteroids (mean daily dose 7.0 mg, range 1–10 mg). Patients were divided into those taking steroids (RA+;  $n = 35$ ) and those not taking steroids (RA-;  $n = 71$ ). One hundred twelve control subjects were randomly selected from a population screening program of more than 1,000 women age 45–64 years identified from the patient register of a large general practice (12). Control subjects with serious concurrent illness and those receiving HRT were excluded.

**Treatment.** Patients were randomized to receive either HRT or calcium supplementation. HRT was in the form of continuous transdermal estradiol valerate 50  $\mu\text{g}$  daily, with norethisterone 1 mg daily for 12 days monthly for patients who had an intact uterus (Estrapak 50; Ciba Pharmaceuticals, Horsham, UK). Patients who had had a hysterectomy took transdermal estradiol only. Calcium supplementation was given as 400 mg elemental calcium daily (calcium lactate-gluconate and calcium carbonate; Sandoz, Frimley, UK). All patients taking SAARDs or steroids had begun this therapy at least 6 months previously and were receiving stable doses.

**Assessments. Biochemistry.** Total osteocalcin was measured using a new human specific immunoradiometric assay (ELSA-OSTEO; Cis Biointernational, Bagnols, France) which recognizes a large N-terminal mid-fragment in addition to the intact molecule (13). Intra- and interassay coefficients of variation were 3.1% and 1.8%, respectively. Bone-specific alkaline phosphatase (BALP) was assayed with a direct immunoradiometric assay using 2 isoenzymes directed against the bone isoenzyme (Ostase; Hybritech, San Diego, CA). The cross-reactivity of BALP with the liver isoenzyme is ~16%, and the intra- and interassay coefficients of variation were 6.4% and 6.8%, respectively. Deoxyypyridinoline (DPyr), measured using high performance liquid chromatography, is a lysine-derived collagen cross-link that is almost exclusive to bone and dentin, and its urinary excretion is reflective of bone degradation. The intraassay coefficient of variation for DPyr was <10%. A second urinary measure, CrossLaps (XL) (Osteometer, Ballerup, Denmark), is a new and sensitive marker of bone resorption that measures a peptide fragment of type I collagen breakdown using an enzyme-linked immunosorbent assay (14). Intra- and interassay coefficients of variation for XL were 11.7% and 13.8%, respectively. Urinary markers were expressed after standard correction for urinary creatinine excretion. Since corticosteroids may also increase muscle catabolism, urinary markers were also corrected for serum creatinine when comparing RA+ patients with RA- patients.

Ninety-four patients had serum available for OC and BALP measurements at 3 months after study entry. Urine was available for baseline DPyr analysis in all patients but was available for XL analysis in only 69 patients, of whom 42 also had 3-month samples. Characteristics of this group of 69 patients were not different from those of the whole cohort.

**Bone density.** Bone mineral density (BMD) of the spine and proximal femur was measured at study entry and at 12 months and 24 months, using dual x-ray absorptiometry. At our institution, short-term reproducibility of repeated measurements in healthy women on different days was 0.9% and 1.5% for the spine and the femur, respectively.

**Disease activity.** RA disease activity was measured with a modified Ritchie articular index (RAI) (15) using 24 joints, and the erythrocyte sedimentation rate (ESR). A Health Assessment Questionnaire (HAQ) (16) measured disability at entry.

**Statistics.** Baseline intergroup analyses were performed using Student's *t*-test and the Mann-Whitney U test. Any non-normal data were logarithmically transformed. Regression equations were used for analyses with confounding variables. Longitudinal analysis was performed on an intent-to-treat basis, using Student's *t*-test and analysis of variance for confounding variables.

## RESULTS

Table 1 shows baseline data on patients taking corticosteroids (RA+ group), patients not taking steroids (RA- group), and controls. The mean OC level was significantly lower in both RA groups compared with controls. Values did not change significantly when corrected for age and weight. Although OC levels tended to be lower in RA+ patients compared with RA- patients, this difference was not significant. There was no effect of cumulative steroid dose on OC level. DPyr and XL levels, corrected for urinary creatinine, were similar in controls and RA- patients but significantly elevated in RA+ patients compared with both RA- patients and controls. After further correction for serum creatinine, the difference in DPyr and XL between RA groups became more significant ( $P < 0.01$ ).

Forty-five patients receiving HRT and 49 patients receiving calcium had serum available for analysis after 3 months; urine was available from 20 patients taking HRT and 22 patients taking calcium. Figure 1 shows the effects of HRT and calcium on changes in serum OC, BALP, and urinary XL levels over 3 months. There were no changes in the level of either OC or BALP. To exclude the possibility of a late effect on OC levels, 6-month measurements were performed, but mean changes were not different from those found at the 3-month assessment, i.e., a mean  $\pm$  SEM increase of  $1.9 \pm 5.4\%$  at 6 months compared with baseline in the group as a whole ( $n = 29$ ) and of  $8.9 \pm 6.4\%$  at 6 months in the steroid-treated subgroup ( $n = 10$ ). Mean XL levels fell significantly with HRT treatment compared with calcium treatment, both in RA- patients (mean 54.9% versus 9.2%;  $P < 0.001$ ) and RA+ patients (29.0% versus 6.7%;  $P < 0.05$ ). There was no significant difference in the percentage decrease of XL levels between RA- patients and RA+ patients, although the power of this comparison was weakened by small cohort sizes. The mean daily

**Table 1.** Baseline characteristics and biochemical markers in the rheumatoid arthritis (RA) patients taking corticosteroids (RA+), RA patients not taking corticosteroids (RA-), and controls\*

	RA+ (n = 35)	RA- (n = 71)	Controls (n = 112)
<b>Clinical features</b>			
Age, years	57.2 ± 5.2†	55.7 ± 5.1	54.8 ± 5.3
Years since menopause	8.6 ± 6.4	7.7 ± 6.3	8.6 ± 7.0
Disease duration, years	11.4 ± 10.2	8.6 ± 6.9	-
ESR, mm/hour	41.7 ± 27.4	31.4 ± 20.5	-
RAI, 0-72 scale	12.6 ± 10.8	9.3 ± 8.6	-
HAQ, 0-3 scale	1.6 ± 1.5	1.3 ± 0.9	-
<b>Biochemical markers</b>			
OC, µg/liter	18.0 ± 8.8‡	20.3 ± 7.1†	23.4 ± 10.3
BALP, µg/liter	13.8 ± 5.2	12.0 ± 4.1	12.8 ± 4.7
DPyr, nM/mM creatinine	21.5 ± 17.1†	8.2 ± 11.2§	11.7 ± 6.1
CrossLaps, µg/mM creatinine	368 ± 192‡	276 ± 181§	252 ± 158

\* Values are the mean ± SD. ESR = erythrocyte sedimentation rate; RAI = Ritchie articular index; HAQ = Health Assessment Questionnaire (0 = no disability; 3 = severe disability); OC = osteocalcin; BALP = bone-specific alkaline phosphatase; DPyr = deoxypyridinoline.

†  $P < 0.05$  versus controls.

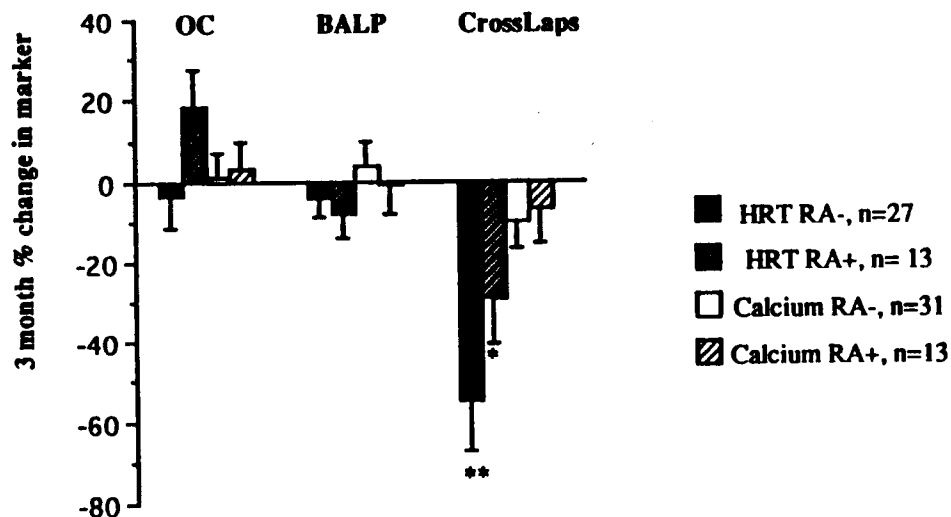
‡  $P < 0.01$  versus controls.

§  $P < 0.05$  versus RA+ group.

prednisolone dose did not change significantly over 2 years (6.3 mg at 1 year, 6.5 mg at 2 years).

After adjustment for number of years since onset of menopause, disease activity measures were found to be inversely correlated with OC level (RAI  $r = -0.32$ ,  $P = 0.003$ ; ESR  $r = -0.23$ ,  $P = 0.04$ ), as was the HAQ score ( $r = -0.23$ ,  $P = 0.04$ ). Disease activity was not significantly correlated with DPyr or XL.

There were no significant correlations between markers of bone formation, either at baseline or after treatment, and baseline or subsequent change in BMD. After 24 months, mean spinal BMD had increased by 3.15% (95% confidence interval [95% CI] 0.35, 4.95) in the HRT group and decreased by 1.1% (95% CI -2.74, 0.64) in the calcium-treated group. There was a significant correlation between the change



**Figure 1.** Changes in levels of bone markers following treatment with hormone replacement therapy (HRT) or calcium in rheumatoid arthritis (RA) patients not taking steroids (RA-) and RA patients taking steroids (RA+). \*\* =  $P < 0.001$  versus calcium RA- group; \* =  $P < 0.05$  versus calcium RA+ group. For CrossLaps, n = 9, 11, 10, and 12 for the HRT RA-, HRT RA+, calcium RA-, and calcium RA+ groups, respectively. OC = osteocalcin; BALP = bone-specific alkaline phosphatase.

in XL after 3 months and the change in BMD after 2 years (for the spine,  $r = -0.37$ ,  $P < 0.01$ ; for the femur,  $r = -0.29$ ,  $P < 0.05$ ).

## DISCUSSION

This study examined markers of bone formation and resorption in a large heterogeneous cohort of female RA patients and demonstrated 2 differing patterns of bone metabolism. First, in the patients with chronic RA not treated with steroids, bone resorption is not generally increased, but the low levels of OC suggest that bone formation is reduced. Thus, bone metabolism in RA appears to be uncoupled. Second, in the case of patients treated with low-dose steroids, this uncoupling appears to be more pronounced since resorption markers are elevated while OC remains depressed.

Previous studies of bone markers in RA have yielded conflicting results (4–6). In histomorphometric studies, RA has been associated with reduced formation and OC levels correlate with mineral apposition rates (6,17). It is uncertain as to why the findings of several of these studies are inconsistent. Clearly, differences in techniques may be responsible. Another possible explanation lies in the effect of cytokines on bone metabolism. Many cytokines inhibit bone formation *in vitro* and *in vivo* (18,19), in accordance with our findings of an inverse relationship between disease activity and OC. However, there may be a biphasic effect of disease activity on OC; Ellies et al found that short-term exposure of fetal rat calvariae to interleukin-1 $\alpha$  led to stimulation of bone formation, whereas continuous exposure resulted in reduced formation (20). Thus, different phases of osteoblastic activity may occur during different phases of disease activity.

OC levels were not significantly lower in RA+ patients compared with RA- patients, probably reflecting the low steroid doses used. In other studies in which low OC levels were shown, much higher steroid doses were used and a dose-dependent effect was demonstrated (21,22). There was no dose effect in our group, but the dose range was narrow (1–10 mg/day).

Neither marker of collagen breakdown was increased in the RA- group. DPyr, reflecting bone degradation, has previously been reported to be elevated in RA (23), whereas studies of XL have not been reported. Importantly, both markers were elevated in patients taking steroids, demonstrating the deleterious effect of steroids on bone resorption, even with low

doses, and probably accounting for the 7% reduction in bone density previously seen in this group (2).

With HRT there was a reduction in collagen cross-link excretion in both the RA+ and the RA- groups. However, OC and BALP did not change with HRT, in contrast to its effects in normal postmenopausal women (24). Reasons for this discrepancy are unclear, but it is important to note that active disease, which depresses OC levels, was itself modestly suppressed by HRT (25). Thus, the expected decline in OC levels in postmenopausal women given HRT may be partially negated by its positive effect on disease activity, and therefore on the osteoblast, in patients with RA. One other published study showed that oral estradiol treatment led to a reduction in OC in 20 RA patients (26), but this group had initial OC levels in the upper normal reference range and did not show any improvement in disease activity.

Current interest in biochemical markers includes their potential value in predicting changes in bone mass. We found that changes in resorption markers after 3 months of treatment with HRT predicted 2-year changes in spinal BMD and, to a lesser extent, femoral BMD. Further prospective studies will be needed to confirm a role for these markers as an indicator of response to treatment.

This study suggests that bone metabolism may be uncoupled in postmenopausal women with RA. Bone formation, as measured by OC, is reduced partly as a function of disease activity. The addition of low-dose corticosteroid treatment may increase bone resorption, further exaggerating any uncoupling. HRT reduces resorption in RA, irrespective of steroid usage, confirming its clinical value in the treatment of RA in postmenopausal women (8,27).

## ACKNOWLEDGMENTS

We are grateful for the help and participation of all the respective clinicians at the following rheumatology departments: Chase Farm Hospital, Homerton Hospital, The Royal London Hospital, St Bartholomew's Hospital, and Whipps Cross Hospital (all in London).

## REFERENCES

1. Spector TD, Hall GM, McCloskey EV, Kanis JA: Risk of vertebral fracture in women with rheumatoid arthritis. *Br Med J* 306:558, 1993
2. Hall GM, Spector TD, Griffin AJ, Jawad ASM, Hall ML, Doyle DV: The effect of rheumatoid arthritis and steroid therapy on bone density in postmenopausal women. *Arthritis Rheum* 36: 1510–1516, 1993

3. Laan RFJM, Buijs WCAM, Verbeek ALM, Draad MP, Corstens FHM, van de Putte LBA, van Riel PLCM: Bone mineral density in patients with recent onset rheumatoid arthritis: influence of disease activity and functional capacity. *Ann Rheum Dis* 52:21-26, 1993
4. Marhofer W, Schatz H, Stracke H, Ullmann J, Schmidt K, Federlin K: Serum osteocalcin levels in rheumatoid arthritis: a marker of accelerated bone turnover in late onset rheumatoid arthritis. *J Rheumatol* 18:1158-1162, 1991
5. Butler RC, Davie MWJ, Worsfold M, Sharp CA: Bone mineral content in patients with rheumatoid arthritis: relationship to low-dose steroid therapy. *Br J Rheumatol* 30:86-90, 1991
6. Kroger H, Risteli J, Risteli L, Penttila I, Alhava E: Serum osteocalcin and carboxyterminal propeptide of type I procollagen in rheumatoid arthritis. *Ann Rheum Dis* 52:338-342, 1993
7. Lukert BP, Raisz LG: Glucocorticoid induced osteoporosis: pathogenesis and management. *Ann Intern Med* 112:352-264, 1990
8. Hall GM, Daniels M, Doyle DV, Spector TD: Effect of hormone replacement therapy in rheumatoid arthritis patients treated with and without steroids. *Arthritis Rheum* 37:1499-1505, 1994
9. Sambrook P, Birmingham J, Champion D, Kelly P, Kempler S, Freund J, Eisman J: Postmenopausal bone loss in rheumatoid arthritis: effect of estrogens and androgens. *J Rheumatol* 19:357-361, 1992
10. Lukert BP, Johnson BE, Robinson RG: Estrogen and progesterone replacement therapy reduces glucocorticoid-induced bone loss. *J Bone Miner Res* 7:1063-1069, 1992
11. Ropes MW, Bennett GA, Cobb S, Jacox R, Jessar RA: 1958 revision of diagnostic criteria for rheumatoid arthritis. *Bull Rheum Dis* 9:175-176, 1958
12. Hart DJ, Spector TD: The relationship of obesity, fat distribution and osteoarthritis in women in the general population: The Chingford Study. *J Rheumatol* 20:331-335, 1993
13. Garnero P, Grimaux M, Demiaux B, Preaudat C, Seguin P, Delmas PD: Measurement of serum osteocalcin with a human specific two-site immunoradiometric assay. *J Bone Miner Res* 7:1389-1398, 1992
14. Garnero P, Gineyts E, Riou JP, Delmas PD: Assessment of bone resorption with a new marker of collagen degradation in patients with metabolic bone disease. *J Clin Endocrinol Metab* 79:780-785, 1994
15. Ritchie DM, Boyle JA, McInnes JM, Jasani MK, Dalakos TG, Grievson P, Buchanan WW: Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. *Q J Med* 37:393-406, 1968
16. Fries JF, Spitz P, Kraines RG, Holman HR: Measurement of patient outcome in arthritis. *Arthritis Rheum* 137-145, 1980
17. Compston JE, Vedi S, Mellish RWE, Croucher P, O'Sullivan MM: Reduced bone formation in non-steroid treated patients with rheumatoid arthritis. *Ann Rheum Dis* 48:483-487, 1989
18. Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR: Stimulation of bone resorption and inhibition of bone formation in vitro by human tissue necrosis factors. *Nature* 319:516-518, 1986
19. Ngyen L, Dewhirst FE, Hauschka PV, Stashenko P: Interleukin 1b stimulates bone resorption and inhibits bone formation in vivo. *Lymphokine Cytokine Res* 10:15-19, 1991
20. Ellies LG, Aubin JE: Temporal sequence of interleukin 1-mediated stimulation and inhibition of bone formation by isolated fetal rat calvaria cells in vitro. *Cytokine* 2:430-437, 1990
21. Delmas PD, Malaval L, Arlot ME, Meunier PJ: Serum Gla-protein compared to bone histomorphometry in endocrine diseases. *Bone* 6:339-341, 1985
22. Peretz A, Praet JP, Bosson D, Rozenberg S, Bourdoux P: Serum osteocalcin in the assessment of corticosteroid induced osteoporosis: effect of long and short term corticosteroid treatment. *J Rheumatol* 16:363-367, 1989
23. Gough AKS, Peel NFA, Eastell R, Holder RL, Lilley J, Emery P: Excretion of pyridium crosslinks correlates with disease activity and appendicular bone loss in early rheumatoid arthritis. *Ann Rheum Dis* 53:14-17, 1994
24. Johansen JS, Riis BJ, Delmas PD, Christiansen C: Plamsa BGP: an indicator of spontaneous bone loss and of the effect of estrogen treatment in postmenopausal women. *Eur J Clin Invest* 18:191-195, 1988
25. Hall GM, Daniels M, Huskisson EC, Spector TD: A randomised controlled trial of hormone replacement therapy in postmenopausal rheumatoid arthritis. *Ann Rheum Dis* 53:112-116, 1994
26. Lems WF, van den Brink HR, Gerrits MI, van Rijn HJM, Bijlsma JWJ: Effect of hormone replacement therapy on markers of bone metabolism in RA. *Ann Rheum Dis* 52:835-836, 1993
27. Van den Brink, Lems WF, van Everdingen AA, Bijlsma JWJ: Adjuvant oestrogen treatment increases bone mineral density in postmenopausal women with rheumatoid arthritis. *Ann Rheum Dis* 52:302-305, 1993