Research article

Hormone replacement therapy, calcium and vitamin D_3 versus calcium and vitamin D_3 alone decreases markers of cartilage and bone metabolism in rheumatoid arthritis: a randomized controlled trial [ISRCTN46523456]

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Abstract

This study aimed to evaluate the effects of hormone replacement therapy (HRT), known to prevent osteoporosis and fractures, on markers of bone and cartilage metabolism. Furthermore, we assessed whether changes in these markers corresponded to alterations in bone mineral density and radiographic joint destructions in postmenopausal women with rheumatoid arthritis. Eighty-eight women were randomized to receive HRT, calcium, and vitamin D_3, or calcium and vitamin D_3 alone, for 2 years. Bone turnover was studied by analyzing serum levels of C-terminal telopeptide fragments of type I collagen (CTX-I), C-terminal telopeptide of type I collagen (ICTP), bone sialoprotein, and C-terminal propeptide of type I procollagen (PICP) and cartilage turnover by urinary levels of collagen type II C-telopeptide degradation fragments (CTX-II) and cartilage oligomeric matrix protein (COMP) in serum. Treatment with HRT resulted in decrease in CTX-I ($P < 0.001$), ICTP ($P < 0.001$), PICP ($P < 0.05$), COMP ($P < 0.01$), and CTX-II ($P < 0.05$) at 2 years. Reductions in CTX-I, ICTP, and PICP were associated with improved bone mineral density. Of the markers tested, CTX-I reflected bone turnover most sensitively; it was reduced by $53 \pm 6\%$ in the patients receiving HRT. Baseline ICTP ($P < 0.001$), CTX-II ($P < 0.01$), and COMP ($P < 0.05$) correlated with the Larsen score. We suggest that biochemical markers of bone and cartilage turnover may provide a useful tool for assessing novel treatment modalities in arthritis, concerning both joint protection and prevention of osteoporosis.

Keywords: bone turnover, cartilage turnover, hormone replacement therapy, osteoporosis, rheumatoid arthritis

Introduction

Rheumatoid arthritis is characterized by cartilage destruction, bone erosions, periarticular osteoporosis, and generalized bone loss resulting in increased prevalence of osteoporotic fractures [1,2]. Some of the disease mechanisms responsible for focal bone loss may be similar to processes of generalized osteoporosis and associated with osteoclast activation [3-5].

Skeletal maintenance occurs by a tightly coupled process of bone remodeling consisting of a process of bone resorption by the osteoclasts followed by deposition of new bone by the osteoblasts. Estrogen deficiency is known to increase bone remodeling and the sustained increase in bone turnover induces a faster bone loss. Hormone replacement therapy (HRT) is known to restore this imbalance [6] and reduce the incidence of spinal and peripheral

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BMD = bone mineral density; BSP = bone sialoprotein; COMP = cartilage oligomeric matrix protein; CTX-I = C-terminal telopeptide fragments of type I collagen; CTX-II = C-terminal telopeptide fragments of type II collagen; DMARD = disease-modifying antirheumatic drug; E_2 = estradiol; ELISA = enzyme-linked immunosorbent assay; ESR = erythrocyte sedimentation rate; HRT = hormone replacement therapy; ICTP = C-terminal telopeptide of type I collagen; PICP = C-terminal propeptide of type I procollagen; RA = rheumatoid arthritis.

fractures in healthy women [7,8] and also to improve bone mass in women with rheumatoid arthritis (RA) [9,10]. Expression of estrogen receptors has been demonstrated in osteoblastic cells [11], osteoclastic cells [12], and human articular chondrocytes [13]. Estrogen decreases osteoclast formation and activity and increases apoptosis of osteoclasts [14,15]. Furthermore estrogen also seems to have a stimulatory effect on bone formation by the osteoblasts [16]. Combined, these two effects are responsible for the bone-protective effects of estrogen and they also explain why women experience an accelerated bone loss after the menopause.

Generalized bone loss in postmenopausal women with RA will occur as a result of decreased estrogen levels accelerating bone turnover and systemic bone loss and by the inflammatory processes resulting in systemic increase of several cytokines shown to up regulate systemic bone turnover. In addition, bone loss also takes place focally as a consequence of the arthritic disease process. Markers of bone turnover provide an integrated measure of systemic turnover, and several studies have demonstrated significant elevations in, especially, resorption markers in RA [17-20]. Elevated bone resorption markers are associated with active progressive disease and decrease in bone mineral density (BMD) [18-20].

We have recently reported that treatment with HRT for 2 years in postmenopausal women with RA significantly improved BMD and also indicated a protective effect on joint destruction [10]. The aim of this randomized, controlled trial was to assess the effect of HRT in postmenopausal RA on biochemical markers of bone and cartilage turnover, the correlations between the markers and bone mass and joint damage, and the associations between changes in biochemical markers and changes in BMD and joint destruction at 2 years. The a priori assumption was that the HRT would induce a significant reduction of not only bone turnover but also cartilage turnover, indicating a structure-modifying therapeutic effect of HRT in RA.

We found that HRT reduced markers of both bone and cartilage metabolism and that the decrease in markers of bone turnover was associated with BMD gain. The type I collagen degradation marker ICTP (C-terminal telopeptide of type I collagen) and the cartilage markers CTX-II (C-terminal telopeptide fragments of type II collagen) and cartilage oligomeric matrix protein (COMP) were associated with the Larsen score at baseline. Of the markers tested, CTX-I (C-terminal telopeptide fragments of type I collagen) reflected bone turnover most sensitively.

Materials and methods
Patients and trial design
Five hundred ninety-two female patients with RA, aged 45–65 years, were identified from rheumatology clinic patient registers in Göteborg and Borås, Sweden. They were invited by mail to participate in a 2-year clinical randomized, single blind, controlled study. The women had to be postmenopausal, defined as not having menstruated in the previous year and having a serum follicle-stimulating hormone (FSH) level >50 IU/l (Diagnostic Products Corporation, Los Angeles, CA, USA). Of the women who were sent the letter inviting them to take part, 81% (478/592) replied. The patients had to have active disease that met at least two of the following criteria: at least six painful joints, at least three swollen joints, an erythrocyte sedimentation rate (ESR) $\geq$ 20 mm per hour, and C-reactive protein concentration $\geq$ 10 mg/l, and they had to fulfill the American Rheumatism Association 1987 revised criteria for adult RA [21]. A maximum daily dose of 7.5 mg of prednisolone was accepted and intra-articular and intramuscular glucocorticosteroid injections were allowed during the study period. The patients were not receiving and had not used in the preceding 2 years drugs affecting bone metabolism (HRT or bisphosphonates), except calcium and vitamin D_3, which were allowed. They had no contraindications to HRT. Three hundred ninety (390/478) of the women could not participate for the following reasons: seventy-two of the women were not postmenopausal and 19 did not fulfill the diagnostic criteria for RA, 159 had been treated with HRT during the preceding 2 years, 26 had a history of deep venous thrombosis or embolism, 23 of cancer of the breast, uterus, or ovaries, 18 had started disease-modifying antirheumatic drugs (DMARDs) or glucocorticosteroid therapy within the previous 3 months or had language problems or had moved to other parts of Sweden, 6 were treated with bisphosphonates, and 67 did not want to participate. Eighty-eight (23%) of the probands entered the study. Patients who dropped out were included in calculations until their withdrawal.

All patients gave their informed consent and the Ethics Committee at the Göteborg University approved the study.

Treatment
Forty-one patients were allocated to the HRT group and 47 to the control group by simple randomization by an independent research nurse. All patients were treated with a daily dose of 500 mg calcium and 400 IU vitamin D_3. Women in the HRT group who were more than 2 years past the menopause were given continuous treatment with 2 mg estradiol (E_2) plus 1 mg norethisterone acetate daily (23 patients). Patients who had had a hysterectomy were given just 2 mg E_2 (4 patients) and the remaining women were given 2 mg E_2 for 12 days, followed by 2 mg E_2 plus 1 mg norethisterone acetate for 10 days, followed by 1 mg E_2 for

6 days (14 women). The gynecologists examined all patients at entry into the study and after 12 and 24 months. The investigators in the rheumatology departments were blinded to the identity of the treatment. Regular medication for RA could be altered by the clinician but not by the investigator.

Assessment of outcome variables

Venous blood and urine samples were obtained at study entry and after 12 and 24 months; they were taken in the morning after an overnight fast. The samples were stored at -70°C until the time of analysis. Serum and urine samples from all time points were analyzed simultaneously except the ESR, which was measured consecutively.

Carboxy-terminal telopeptide fragments of type I collagen

Serum levels of CTX-I derived from bone resorption were measured by a one-step ELISA (Nordic Bioscience A/S, Herlev, Denmark) using two monoclonal antibodies specific for a β-aspartate form of the epitope EKAHDGGR derived from the carboxy-terminal telopeptide region of type I collagen $\alpha 1$ chain [22,23]. The detection limit was 0.01 ng/ml [23]. Intra- and inter-assay coefficients of variation of the serum CTX-I assay were 5.4 and 6.2% respectively. All samples were measured in duplicate and samples from the same patient were measured on the same ELISA plate. Samples were re measured if coefficients of variation exceeded 15%.

Carboxy-terminal telopeptide of type I collagen

Radioimmunoassay was used for the quantitative determination in serum of the bone resorption marker ICTP (Orion Diagnostica, Espoo, Finland). The detection limit of the test was 0.5 µg/l and the intra-assay and interassay coefficients of variation were <8% according to the manufacturer and <6% in our laboratory.

Bone sialoprotein

An inhibition ELISA with a polyclonal antiserum raised against human bone sialoprotein (BSP) was used for measurement in serum of BSP, a marker reflecting bone turnover [24]. The detection limit of the test was 2.5 ng/ml and the intra-assay and interassay coefficients of variation were <10%.

Carboxy-terminal propeptide of type I procollagen

Radioimmunoassay was used for the quantitative determination in serum of the collagen type I turnover marker PICP (C-terminal propeptide of type I procollagen) (Orion Diagnostica). The detection limit of the test was 1.2 µg/l and the intra-assay and interassay coefficients of variation were <7% according to the manufacturer and <5% in our laboratory.

Collagen type II degradation fragments

Urinary levels of CTX-II, reflecting cartilage breakdown, were measured by a new competitive ELISA (CartiLaps; Nordic Bioscience A/S, Herlev, Denmark) based on a mouse monoclonal antibody raised against the EKGPDP sequence of human type II collagen C-telopeptide. This sequence is found exclusively in type II collagen and not in other collagens, including type I collagen or other structural proteins. The detection limit of the test was 0.15 ng/ml [25]. Intra-assay and interassay coefficients of variation of the urine CTX-II assay were 7.1% and 8.4% respectively. Urinary CTX-II was corrected by the urinary creatinine concentration measured by a standard colorimetric method and expressed as the ratio of CTX-II (ng) to urinary creatinine (mmol).

Cartilage oligomeric matrix protein

The cartilage-turnover marker COMP was measured in serum by a sandwich ELISA based on two monoclonal antibodies directed against separate antigenic determinants on the human COMP molecule (AnaMar Medical, Lund, Sweden). The detection limit of the test was 0.1 U/l and the intra-assay and interassay coefficients of variation were <5%. The serum concentrations of COMP obtained by this assay are highly correlated with serum levels obtained by the original inhibition assay (*r* values >0.9 in RA samples) [26] (Saxne T and Heinegård D, unpublished).

Estradiol

E_2 levels in serum were measured (around 12 hours after tablet intake) at baseline and yearly thereafter using radioimmunoassay (Clinical Assays™ DiaSorin, Vercelli, Italy). The detection limit of the test was 18 pmol/l.

Bone mineral density

BMD in the left forearm, left hip, and lumbar spine was measured at study entry and at 12 and 24 months by dual-energy x-ray absorptiometry (DXA) with Hologic QDR-4500A® (Hologic, Bedford, MA, USA).

Radiographs

Radiographs of the hands, wrists, and distal part of the feet were obtained at baseline and after 12 and 24 months. Forty joints in the hands and feet were scored (in the hands, proximal interphalangeal joints of digits 1–5, metacarpophalangeal joints of digits 1–5, wrist areas 1–4; and in the feet, the interphalangeal joints of digit 1 and the metatarsophalangeal joints of digits 1–5). The radiographs were masked for identity and sequence and they were evaluated by Dr Arvi Larsen [27], who was unaware of the treatments of the patients. Each joint was scored from 0 (normal) to 5 (maximal destruction). The scores for each patient were summarized and then divided by the number of examined joints to give the patient's mean Larsen score, ranging from 0 to 5.

Disease Activity Score 28
DAS 28 [28] was assessed at all check points, calculated by the following formula: DAS 28 = 0.56 TJC$^{1/2}$ + 0.28 SJC$^{1/2}$ + 0.70lnESR + 0.014GH, where TJC gives the tender joint count, SJC, the swollen joint count, and GH, the patient's assessment of general health using a Visual Analogue Scale of 100 mm.

Statistical analysis
The primary end points of the original study were radiographic progression of joint damage and change in BMD over the 2-year observation period [10]. Biochemical marker measurements were added as secondary end points. For power calculation concerning the number of patients needed to detect a significant difference of BMD between study groups at the significance level 0.05, a two-tailed test with 90% power was conducted. The number of patients included in the trial was well sufficient. The data found for the biomarkers were not normally distributed, and nonparametrical tests were therefore used. Comparisons between the groups were made using the Mann–Whitney U test, and changes within the treatment groups by the Wilcoxon rank sum test. Associations between biochemical markers, BMD, and joint destruction were assessed by Spearman's rank correlation test. A χ^2 test was used to compare proportions. To account for the multiple comparisons made in the statistical assessment of the data, actual P values are shown. All tests were two-tailed and $P \leq 0.05$ was considered statistically significant.

Results
Patient population
The two patient groups were comparable with respect to all variables tested at baseline. The age (years) in the HRT group was 57.0 ± 5.5 (mean ± SD) and in the controls, 58.1 ± 4.7, and the disease durations (years) were 16.4 ± 11.9 and 15.5 ± 11.7, respectively. Thirty-four (83%) of the women in the HRT group had positive tests for rheumatoid factor, compared with 40 (85%) of the controls. Prior drug use was similar in the two groups at the start of the study, with disease-modifying antirheumatic drug (DMARD) use in 34 patients (83%) of the HRT group and 37 (79%) of the controls ($P = 0.58$). Ten (24%) patients in the HRT group and 9 (19%) in the control group were treated with oral glucocorticosteroids ($P = 0.55$) at a mean dosage of 4.6 mg of prednisolone, and 17 (44%) and 13 (28%), respectively, were treated with methotrexate ($P = 0.14$). No patient used biologic agents, since they were not available when the study started. The proportions of patients treated with DMARDs, methotrexate, and corticosteroids were equal in the HRT and control groups at all check points during the study. No significant differences between the treatment groups were found regarding change in DMARDs, or the amounts of corticosteroids injected intra-articularly and intramuscularly. For further information, please see a previ-

ous report [10]. There were no significant differences regarding ESR, E$_2$, or biochemical markers of bone and cartilage metabolism between the two study groups at baseline (Table 1).

Six (15%) patients in the HRT group and 2 (4%) in the control group withdrew from the study before completing the 2 years (Table 2). No serious side effects were observed. For some patients, incomplete sample sets were available for analysis of biochemical markers. The number of samples available for each analysis is presented in Table 1.

The impact of HRT on biochemical markers of bone and cartilage turnover
As reported previously, BMD increased significantly, by 3.6% in the forearm, 4.0% in the total hip, and 7.1% in the lumbar spine in the HRT group [10]. Furthermore, there was an indication of a joint-protective effect of the HRT treatment [10]. The results of the bone and cartilage biochemical marker analyses are shown in Table 1.

Markers of bone turnover
HRT caused a pronounced decrease in the collagen type I degradation marker, CTX-I, both when the HRT and control groups were compared ($P < 0.001$) and within the HRT group ($P < 0.001$) (Fig. 1a). CTX-I in the HRT group was reduced by 62 ± 5% (mean ± SEM) after 12 months and 53 ± 6% after 24 months. In the control group a decrease of 3 ± 13% and an increase of 12 ± 13% was observed after 12 and 24 months, respectively.

HRT resulted also in a significant ($P = 0.035$) but less pronounced reduction in the other collagen type I degradation product, ICTP. The average percentage reduction of this marker was 5 ± 7% after 12 months and 5 ± 5% after 24 months in the HRT group. A significant increase ($P = 0.002$) of ICTP was found in the controls, by an average of 21 ± 6% after 12 months and 31 ± 10% after 24 months.

BSP, which is a bone-specific protein enriched in the cartilage–bone interface, increased significantly ($P = 0.023$) in the control group but remained unchanged in the HRT group.

The marker of bone formation, PICP, decreased significantly in comparison with the controls ($P = 0.005$) as well as within the HRT group ($P < 0.001$) by the end of the first year. After the second year, a significant decrease was found within the HRT group ($P = 0.021$) in comparison with baseline values. PICP decreased by 23 ± 4% in the first year and by 10 ± 4% the second year in the HRT-treated women.

Table 1

The impact of hormone replacement therapy (HRT) on biochemical markers of bone and cartilage metabolism

		At study entry	At 12 months	At 24 months
CTX-I (ng/ml)	HRT	0.59 ± 0.37 (35)	0.21 ± 0.15 (32) [†††,‡‡‡]	0.25 ± 0.16 (33) [†††,‡‡‡]
	Controls	0.63 ± 0.34 (46)	0.53 ± 0.41 (46) [‡]	0.66 ± 0.66 (42)
ICTP (µg/l)	HRT	5.1 ± 2.1 (35)	4.7 ± 2.2 (29) [††,‡]	4.9 ± 2.4 (33) [†††,‡]
	Controls	4.6 ± 1.7 (44)	5.8 ± 3.4 (46) [‡]	6.5 ± 5.4 (44) [‡‡]
BSP (ng/ml)	HRT	119.3 ± 39.6 (41)	126.8 ± 34.1 (33)	127.6 ± 47.8 (34)
	Controls	126.7 ± 35.7 (47)	139.5 ± 46.5 (47) [‡]	143.1 ± 45.3 (45) [‡]
PICP (µg/l)	HRT	132.8 ± 40.0 (34)	104.0 ± 26.9 (29) [††,‡‡‡]	121.0 ± 31.9 (34) [‡]
	Controls	133.1 ± 42.7 (44)	132.6 ± 50.2 (46)	133.2 ± 40.0 (44)
COMP (U/l)	HRT	11.0 ± 2.6 (39)	10.1 ± 2.9 (33) [†,‡]	9.8 ± 2.7 (34) [††,‡‡]
	Controls	11.3 ± 3.2 (47)	11.6 ± 2.8 (47)	11.8 ± 2.7 (45)
CTX-II (ng/mmol)	HRT	1.1 ± 1.3 (41)	0.8 ± 0.8 (36)	0.9 ± 1.1 (34) [‡]
	Controls	1.2 ± 1.4 (44)	1.3 ± 1.5 (46)	1.1 ± 0.9 (44)
Estradiol (pmol/l)	HRT	47.7 ± 47.9 (31)	177.6 ± 139.4 (25) [†††,‡‡‡]	176.1 ± 124.0 (31) [†††,‡‡‡]
	Controls	37.2 ± 25.5 (40)	38.3 ± 33.2 (41)	37.8 ± 39.2 (38)
ESR (mm)	HRT	30.8 ± 19.1 (41)	29.0 ± 18.8 (35)	24.3 ± 13.1 (35) [†,‡‡]
	Controls	26.5 ± 15.1 (46)	27.4 ± 19.8 (45)	26.3 ± 17.5 (44)
DAS 28	HRT	5.2 ± 1.0 (41)	4.4 ± 1.1 (35) [‡‡‡]	3.9 ± 1.0 (35) [†,‡‡‡]
	Controls	5.3 ± 1.0 (46)	4.8 ± 1.3 (45) [‡‡]	4.5 ± 1.1 (44) [‡‡‡]

Values are means ± SD. Numbers of patients with available data are shown in parentheses. [†]$P <0.05$ for the comparison with controls from baseline with respects to differences; [††]$P < 0.01$ for the comparison with controls from baseline with respects to differences; [†††]$P < 0.001$ for the comparison with controls from baseline with respects to differences; [‡]$P < 0.05$ for the comparison with baseline with respects to differences; [‡‡]$P < 0.01$ for the comparison with baseline with respects to differences; [‡‡‡]$P < 0.001$ for the comparison with baseline with respects to differences. BSP, bone sialoprotein; COMP, cartilage oligomeric matrix protein; CTX-I, type I collagen C-telopeptide fragments; CTX-II, type II collagen C-telopeptide; DAS, Disease Activity Score 28 [28]; ESR, erythrocyte sedimentation rate; ICTP, C-terminal telopeptide of type I collagen, PICP, C-terminal propeptide of type I procollagen.

Table 2

Reasons for withdrawal from the study

|	Patients receiving HRT		Controls	
Year	Reason	No.	Reason	No.
First year	Hyperperspiration	1		
	Hyperparathyroidism	1		
	Nausea	2		
	Nausea and weight gain	1		
Second year	Overlap syndrome	1	Started HRT	1
			Cancer of the thyroid	1
Total		6		2

HRT, hormone replacement therapy.

Markers of cartilage turnover
The HRT group presented a marked decrease in serum lev- els of COMP, both between the HRT and control group (P = 0.003) and within the HRT group (P = 0.003). The

Figure 1
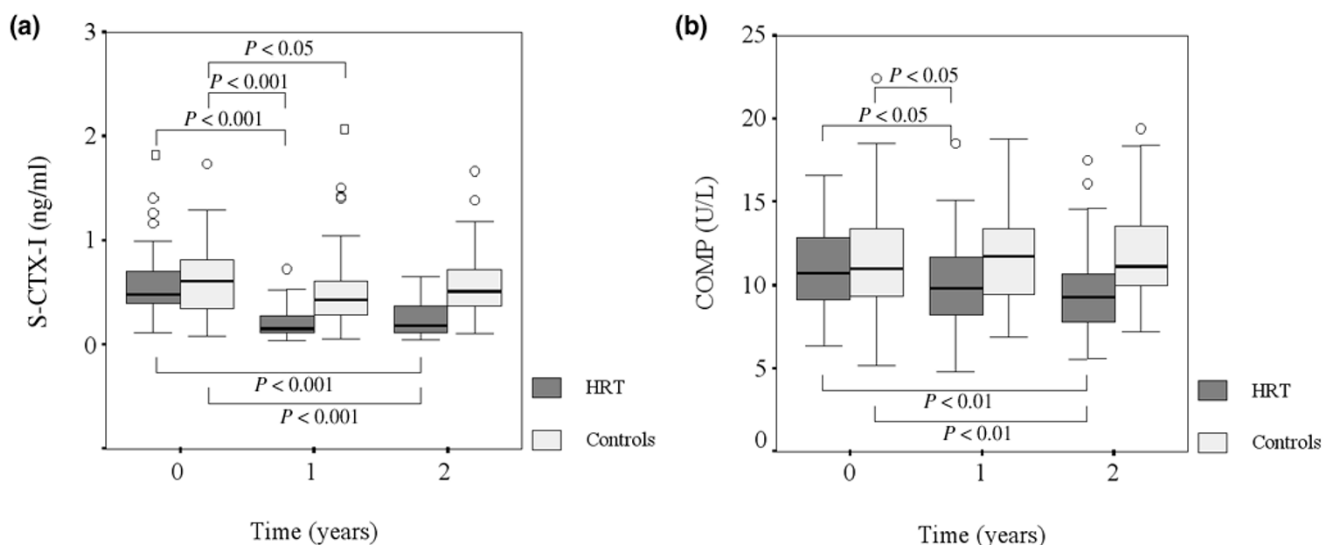
The effects of hormone replacement therapy (HRT) on serum markers of bone and cartilage metabolism. **(a)** Effect of HRT on serum (S) levels of type I collagen C-telopeptide (CTX-I). **(b)** Effect of HRT on serum levels of cartilage oligomeric matrix protein (COMP). Values are the medians (horizontal line), interquartile ranges (box), and ranges (whiskers). Circles (outliers) represent cases with values between 1.5 and 3 box lengths, and boxes (extremes) represent values more than 3 box lengths from the upper or lower edge of the box. Statistical differences between the groups and within each group are given.

percentage reduction was 9 ± 4% in the HRT group, compared with an increase of 7 ± 4% in the controls at 2 years (Fig. 1b).

The urinary marker of cartilage degradation, CTX-II, had decreased significantly at 2 years within the HRT group ($P = 0.023$) in comparison with baseline values.

Correlations at baseline
The correlations at baseline are shown in Table 3. CTX-I was inversely associated with BMD in both the forearm ($P = 0.011$) and the total hip ($P = 0.024$) and was positively associated with ICTP ($P = 0.001$) and PICP ($P < 0.001$).

ICTP, besides showing a positive correlation with CTX-I, was inversely correlated with BMD in the forearm ($P = 0.029$) and total hip ($P = 0.003$) and was positively correlated with ESR ($P = 0.013$), CTX-II ($P < 0.001$) and the Larsen score ($P < 0.001$).

PICP, in addition to its strong correlation with CTX-I, was inversely correlated with BMD in the forearm ($P = 0.017$) and was positively correlated with COMP ($P = 0.020$).

CTX-II was positively correlated with the Larsen score ($P = 0.001$) and ESR ($P = 0.018$) as well as with ICTP.

COMP, in addition to its correlation with PICP, was also correlated with BMD in the lumbar spine ($P = 0.009$) and with the Larsen score ($P = 0.014$).

Long-term changes in biochemical markers correlated with changes in BMD
The alterations in biochemical markers of bone and cartilage metabolism from baseline to 24 months were correlated with each other and with the changes in BMD and radiological destruction during the same period (Table 4).

A decrease in CTX-I was correlated with increased bone mass in the total hip ($P < 0.001$) and lumbar spine ($P < 0.001$) and with a reduction in ICTP ($P < 0.001$), PICP ($P = 0.005$) and ESR ($P = 0.019$). Because E_2 and bone mass changes were strongly correlated, the partial correlation coefficients adjusting for the effect of E_2 changes were calculated, in order to assess if there were independent correlations between CTX-I and BMD. The coefficients remained significant concerning CTX-I and the total hip (-0.308, $P = 0.023$) and CTX-I and the lumbar spine (-0.280, $P = 0.04$).

A reduction in ICTP was correlated with improved BMD in the lumbar spine ($P = 0.002$) and total hip ($P = 0.027$) and with a decrease in ESR ($P = 0.006$), CTX-II ($P = 0.001$), and COMP ($P = 0.005$), besides the correlation with CTX-I. The correlations between changes in ICTP and BMD in the spine and hip remained significant in the hip (-0.301, $P = 0.023$) but not in the spine (-0.175, $P = 0.194$) after adjustment had been made for the effect of changed E_2 levels.

Table 3

Baseline correlations between biochemical markers of bone and cartilage metabolism, bone mineral density, and radiographic joint destruction

Biochemical marker	ESR	CTX-I	ICTP	BSP	PICP	CTX-II	COMP	BMD, forearm	BMD, total hip	BMD, lumbar spine	Larsen score
ESR	-	0.111	0.280*	0.088	0.026	0.257*	0.095	-0.171	-0.195	-0.100	0.500***
CTX-I	0.111	-	0.366**	0.194	0.401***	0.129	0.099	-0.293*	-0.253*	-0.187	0.182
ICTP	0.280*	0.366**	-	-0.120	0.138	0.472***	0.201	-0.255*	-0.322**	-0.129	0.449***
BSP	0.088	0.194	-0.120	-	0.195	0.047	-0.072	0.100	0.012	0.076	-0.045
PICP	0.026	0.401***	0.138	0.195	-	0.069	0.264*	-0.281*	-0.205	-0.218	0.103
CTX-II	0.257*	0.129	0.472***	0.047	0.069	-	0.160	-0.075	-0.170	0.073	0.361**
COMP	0.095	0.099	0.201	-0.072	0.264*	0.160	-	0.099	0.187	0.281**	0.271*
Estradiol	0.015	-0.077	0.020	0.020	-0.041	0.120	0.171	0.209	0.185	0.255*	0.008

Radiographic joint destruction was assessed using the Larsen score [27]. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. BMD, bone mineral density; BSP, bone sialoprotein; COMP, cartilage oligomeric matrix protein; CTX-I, type I collagen C-telopeptide fragments; CTX-II, type II collagen C-telopeptide; ESR, erythrocyte sedimentation rate; ICTP, C-terminal telopeptide of type I collagen; PICP, C-terminal propeptide of type I procollagen.

Table 4

Correlations between changes over 2 years in biochemical markers of bone and cartilage metabolism and in bone mineral density and radiographic joint destruction

|	ΔESR	ΔCTX-I	ΔICTP	ΔBSP	ΔPICP	ΔCTX-II	ΔCOMP	ΔBMD, forearm	ΔBMD, total hip	ΔBMD, lumbar spine	ΔLarsen score
ΔESR	-	0.276*	0.327**	0.004	-0.031	0.407***	0.188	-0.093	-0.092	-0.145	0.068
ΔCTX-I	0.276*	-	0.535***	0.189	0.348**	0.119	0.184	-0.234	-0.507***	-0.501***	0.083
ΔICTP	0.327**	0.535***	-	0.167	0.040	0.401**	0.338**	-0.239	-0.271*	-0.372**	-0.043
ΔBSP	0.004	0.189	0.167	-	-0.092	-0.029	0.004	-0.056	-0.163	0.032	-0.079
ΔPICP	-0.031	0.348**	0.040	-0.092	-	0.042	0.328**	-0.138	-0.266*	-0.222	0.392**
ΔCTX-II	0.407***	0.119	0.401**	-0.029	0.042	-	0.190	-0.094	-0.055	-0.146	0.052
ΔCOMP	0.188	0.184	0.338**	0.004	0.328**	0.190	-	-0.190	-0.204	-0.157	-0.027
ΔEstradiol	-0.079	-0.373**	-0.301*	-0.092	-0.160	-0.094	-0.243	0.067	0.491***	0.483***	0.063

Radiographic joint destruction was assessed using the Larsen score [27]. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. BMD = bone mineral density; BSP, bone sialoprotein; COMP, cartilage oligomeric matrix protein; CTX-I, type I collagen C-telopeptide fragments; CTX-II, type II collagen C-telopeptide; ESR, erythrocyte sedimentation rate; ICTP, C-terminal telopeptide of type I collagen; PICP, C-terminal propeptide of type I procollagen.

A decrease in PICP was correlated with an increase in BMD in the total hip ($P = 0.030$) and with reduction in COMP ($P = 0.006$) and the Larsen score ($P = 0.002$) in addition to the correlation with CTX-I. The correlation between changes in PICP and BMD in the hip remained significant (-0.260, $P = 0.049$) after adjusting for the effect of changed E_2 levels.

Decrease in CTX-II, in addition to the correlation with ICTP, was also strongly correlated with decreased ESR ($P < 0.001$).

Short-term changes in markers correlated with long-term changes in BMD

We also investigated whether the above markers, which were significantly correlated with the outcome of BMD and the Larsen score also, could be predictive when the short-term changes, from baseline to 12 months, were used instead in the correlation analyses. We found that the changes in serum levels of CTX-I over the first 12 months were inversely correlated with the alteration in BMD in the total hip ($P < 0.001$), lumbar spine ($P < 0.001$), and forearm ($P = 0.050$). The percentage change in CTX-I during

Figure 2
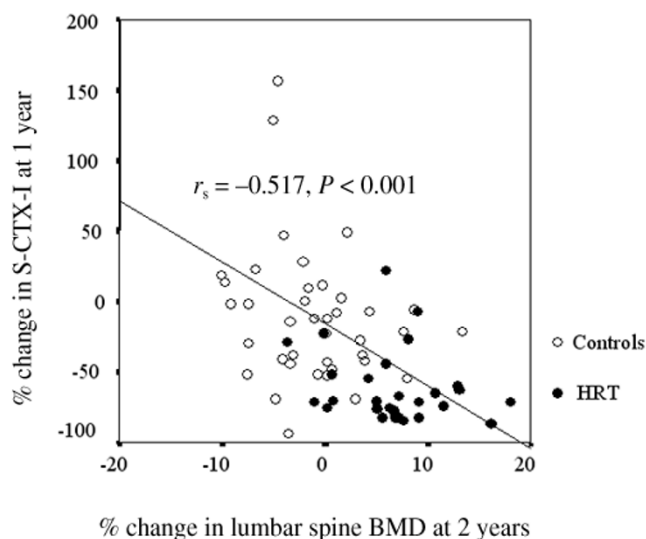
Short-term changes in serum (S) levels of type I collagen C-telopeptide (CTX-I) correlated with long-term changes in bone mineral density (BMD) in the lumbar spine. The percentage change the first year is plotted against the change in BMD after 2 years in the hormone replacement therapy (HRT) and control group. A regression line is inserted showing the significant inverse association.

the first year correlated with the change in BMD in the lumbar spine during the whole trial is shown in Fig. 2.

The short-term change in ICTP was inversely associated with altered bone mass in the forearm ($P = 0.038$) and lumbar spine ($P = 0.023$), and the change in PICP was correlated with the modification in the Larsen score ($P = 0.024$) and inversely with BMD in the total hip ($P = 0.002$), lumbar spine ($P = 0.002$), and forearm ($P = 0.017$).

The short-term change in E_2 was also associated with the 2-year change in bone mass in the total hip ($P = 0.006$) and lumbar spine ($P = 0.007$), so we adjusted the above markers for the effect of alterations in serum levels of estrogen at these measurement sites. The correlations remained significant after adjustment for the influence of estrogen.

Discussion

The main objective of the present study was to analyze the effects of HRT on biochemical markers of both bone and cartilage metabolism in RA in postmenopausal women. This report is the first to show that HRT resulted in reduction of markers of both bone and cartilage turnover in women with RA. We also wanted to evaluate associations between the markers and bone mass and the Larsen score at baseline and to see if the changes in markers could predict BMD and joint destruction. There were significant correlations between several biochemical markers and bone mass and radiological status at entry into the study. Addi-

tionally, decreases in markers of bone metabolism were associated with improved bone mass. These findings support our previous results showing improved bone mass in the forearm, total hip, and lumbar spine, and also indicated a joint-protective effect of HRT in patients with progressive erosive RA [10]. The ESR also decreased and DAS 28 was decreased significantly more in the HRT group than in the controls, as has been shown in more detail in a previous work [10]. However, in view of the recent trials of HRT use among healthy postmenopausal women, showing, for example, an increased risk of cardiovascular events and breast carcinoma [29], there is a need to be cautious about HRT. It is hardly possible to generalize the results from studies of healthy postmenopausal women to patients with RA, a chronic inflammatory disease. In RA patients, the systemic inflammation seems to be more important than traditional risk factors in the development of coronary heart disease, and it may be that HRT use could find better acceptance in RA than among otherwise healthy postmenopausal women, but this issue requires further study [30].

Some limitations of the present study should be mentioned. Corrections have not been made for multiple comparisons, since the findings seem biologically reasonable and in accordance with our a priori hypothesis. Yet, one must be cautious about significances with P values at the <0.05 level, which theoretically could have occurred by chance since quite a lot of tests have been performed. It is also important to take into account that the biochemical markers that we have analyzed are not completely specific for bone or articular cartilage, because minor amounts of these markers may also be released from other tissues. However, we estimate on the basis of previous reports that they reflect bone and cartilage metabolism well enough to be able to follow and assess bone and cartilage turnover [31,32].

Type I collagen comprises more than 90% of the organic bone matrix. Some other tissues also contain type I collagen – for example, skin, tendon, and cornea – but bone has a much higher proportion and a much higher turnover of this protein. Type I collagen has a triple-helix structure. Crosslinking by pyridinoline or deoxypyridinoline occurs between residues on the nonhelical carboxy-terminal or amino-terminal ends, termed telopeptides, and the helical portion of an adjacent collagen [33]. During osteoclastic bone resorption, cathepsin K and other proteases release peptide bound crosslinks, attached to fragments of C-terminal (CTX) or N-terminal (NTX) telopeptides [33,34]. The crosslinks can be measured in the urine and serum as an index of bone resorption. Cathepsin K, which is a major osteoclast-derived protease, directly generates the fragments measured in the CTX-I assay. Another assay specific for fragments of the collagen type I C-telopeptide, ICTP, results primary from nonosteoclastic matrix-metalloprotein-

ase-mediated degradation of type I collagen [34]. In accordance with the specificity of the type I collagen markers, the CTX-I assay has previously been shown to provide a significant response to antiresorptive therapies, including HRT [22,23,33,35]. In addition, strong associations between levels of CTX-I and changes in this marker and subsequent change in BMD have been demonstrated [22]. The CTX-I marker has been less used in RA, but some recent studies have reported that high levels of CTX-I and CTX-II, reflecting bone and cartilage degradation, respectively, were associated with an increased risk of radiological progression in RA [18,20,36].

We found that CTX-I had decreased significantly in the HRT group, by 62% and 53% at 1 and 2 years, respectively. This decrease is comparable to the effect of HRT in healthy postmenopausal women [37]. A small reduction of CTX-I was also noticed in the control group at the end of first year, which possibly could be due to the treatment with calcium and vitamin D_3. CTX-I was inversely correlated with the bone mass in forearm and total hip, and both the 1- and 2-year changes in CTX-I were associated with the 2-year changes in BMD in the lumbar spine and total hip. In addition, the change in CTX-I was associated with a change in serum levels of E_2, suggesting a biological association between the two parameters. The results imply that in RA, also, serum CTX-I provides a good assessment of treatment responses to antiresorptive therapy such as HRT [18,20,36].

We also measured ICTP, which decreased by only 5% in the HRT group, in accord with the findings of previous studies showing similar weak responsiveness of this marker to HRT treatment in healthy postmenopausal women [23]. ICTP increased significantly in the controls, for reasons of which we are not certain. In a previous study of the effect of HRT on ICTP in RA, no change in ICTP was found [38]. These results may be considered to be in accord with the biochemical background of the markers where CTX-I is generated, whereas ICTP is destroyed by cathepsin-K-mediated degradation of the organic bone matrix [34]. At baseline, ICTP was correlated strongly with the Larsen score and to a lesser extent with the ESR, an observation that is in line with findings by others of increased serum levels of ICTP in active RA [39,40].

Type I collagen is synthesized by osteoblasts as a precursor protein termed procollagen I. The carboxy-terminal and amino-terminal ends of procollagen I are removed during fibril formation before type I collagen is incorporated into the bone matrix. This cleavage yields two extension peptides, PICP and procollagen I amino-terminal propeptide (PINP), which are used as markers of bone formation [31,33]. In this study, we analyzed PICP. The marker decreased significantly during the first year in the HRT

group compared with controls, as has previously been found by Lems and co-workers [38]. The reduction was followed by a significant increase within the HRT group during the second year (data not shown), which might indicate an anabolic effect on the osteoblasts by HRT, in line with our previous findings of an increase in insulin-like growth factor 1 in the HRT group [41]. PICP correlated significantly with CTX-I and COMP and inversely with bone mass in the forearm at baseline. The 1- and 2-year decreases in PICP-I were associated with a reduction in COMP levels, improved BMD, and a beneficial effect on the Larsen score. The findings suggest that PICP together with CTX-I reflects the rate of bone turnover, in accord with the findings of Cortet and co-workers [40].

BSP accounts for about 10% of the noncollagenous proteins in bone and is in particular enriched at cartilage–bone interfaces. The function of the protein is not known, although a role in mineralization has been proposed [24,42,43]. In RA, BSP has been shown to be increased in serum, and the concentration of BSP in synovial fluid was correlated with the degree of knee joint damage in RA and was thus considered to reflect tissue breakdown [24]. In a prospective study, it was found that HRT decreased BSP in healthy postmenopausal women [43]. In our study, HRT exerted a suppressive effect, apparent as an increase of BSP in the control group but not in the HRT group. Neither the baseline levels nor the alterations in BSP were associated with bone mass or the Larsen score or its changes during the trial. This contrasts with the results for other bone markers and may be due to the restricted distribution of BSP within the tissue.

Collagen type II is the major structural protein of cartilage, comprising more than 50% of the protein in this matrix [32]. Type II collagen is synthesized by chondrocytes and degraded by proteolytic enzymes secreted by the chondrocytes and synoviocytes, including matrix metalloproteinases. The CTX-II marker derived from degradation of type II collagen was measured as an index of cartilage turnover. Previous studies have shown that CTX-II levels in the urine are elevated in patients with osteoarthritis and RA [20,25,36]. Lehmann and co-workers showed that antiresorptive treatment of postmenopausal women with a bisphosphonate, ibandronate, decreased not only CTX-I but also CTX-II [44]. This indicates a chondroprotective effect of this class of compounds, which has also been suggested by recent *in vitro* [45] and *in vivo* [46] studies. Of interest to our study is the fact that HRT treatment of healthy postmenopausal women has also been shown to be associated with significant lower CTX-II levels, indicating an effect of HRT on cartilage turnover [47]. In the present investigation, CTX-II in the urine decreased significantly within the HRT group, a finding that implies that HRT has a protective effect on cartilage. CTX-II was also corre-

lated with ESR, ICTP, and the Larsen score at entry into the study, and the change in CTX-II at the end of 2 years was associated with the changes in ESR and ICTP, indicating an association with the inflammatory activity and processes of structural damage in the disease.

COMP is a 524-kDa, homopentameric, extracellular-matrix protein and it constitutes 0.5–1% of the wet weight of cartilage and is released from cartilage during the erosive process [26]. Its biological function is still unclear, but findings suggest that COMP may be involved in regulating fibril formation and maintaining the integrity of the collagen network. It was initially found in cartilage [48], but more recently it has also been found to be secreted from other tissues, such as synovial fibroblasts [49]. COMP has been shown to be increased in serum at disease onset in RA patients who developed large-joint destruction [50]. In early RA, high serum levels have recently been found to correlate also with future small-joint damage [51]. Moreover, neutralization of tumour necrosis factor α decreased serum levels of COMP in RA [52]. We show decreasing serum levels of COMP by HRT in this longitudinal study. COMP decreased significantly both within the HRT group and in comparison with the controls. As was discussed in a previous report [5], an explanation of the positive association between COMP and BMD at entry into the study could be due to a strong relation between osteoporosis and severe joint damage with decreased presence of articular cartilage and consequently reduced cartilage turnover.

Both biochemical markers reflecting cartilage metabolism were associated with the Larsen score at baseline but no correlations between changes in the markers and subsequent changes in the Larsen scores were found. One plausible reason for this lack of association may be that the Larsen did not change at all during the trial in about 40% of the patients; this fact reduces the probability of finding any significant associations between changes.

Conclusion
We found in this randomized, controlled trial that treatment with HRT in postmenopausal women with established RA reduced markers of bone turnover as well as of cartilage metabolism. The decrease in bone turnover markers CTX-I, ICTP, and PICP were associated with improved bone mass after 2 years, with CTX-I providing the most sensitive prognostic value. Baseline measures of ICTP and the markers of cartilage turnover, CTX-II and COMP, were correlated with the Larsen score and decreased during HRT treatment. Thus, specific biochemical markers of bone and cartilage turnover may be useful for assessing novel treatment modalities in arthritis, concerning both joint protection and prevention of osteoporosis.

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