

# Evidence for Increased Bone Resorption in Patients With Progressive Knee Osteoarthritis

## Longitudinal Results From the Chingford Study

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**Objective.** Several studies have suggested that increased subchondral bone turnover is a determinant of progression of osteoarthritis (OA). To test this hypothesis, the level of urinary N-terminal type I collagen telopeptides (NTx) and C-terminal type I collagen telopeptides (CTx), which are validated markers of bone resorption, was measured at 3 different time points in a subset of patients from the Chingford study.

**Methods.** The original Chingford study population comprised 1,003 women. From this group, postmenopausal women not receiving any bone-modifying medication who had a baseline knee radiograph and a repeat radiograph 4 years later, and for whom a baseline lumbar spine bone mineral density (BMD) measurement was available, were identified and separated into 4 groups as follows: controls (n = 50), progressive OA (n = 71), nonprogressive OA (n = 36), and osteoporosis (n = 59). NTx and CTx were measured in urine samples collected at baseline, year 1, and year 2.

**Results.** Patient age and years since menopause were similar among groups at baseline. As expected, both body mass index (BMI) and BMD were lowest in patients with osteoporosis. Median resorption marker levels over the 3 time points were 31–87% higher in patients with either progressive OA or osteoporosis than in controls and patients with nonprogressive OA ( $P <$

0.01, except for levels of CTx in patients with progressive OA versus nonprogressive OA). Levels of NTx and CTx did not differ significantly between women with progressive OA (defined either by the presence of osteophytes or by joint space narrowing) and those with osteoporosis or between controls and women with nonprogressive OA. Results were essentially unchanged after adjustment for age, BMI, BMD, and past use of hormone replacement therapy, or when NTx and CTx values at each time point were analyzed separately.

**Conclusion.** Our data demonstrate that bone resorption is increased in patients with progressive knee OA and is not increased in those with nonprogressive knee OA. The increase in bone resorption seen in patients with progressive knee OA is similar to that observed in patients with osteoporosis. Altered bone turnover may be a diagnostic or therapeutic target in patients with progressive OA.

Several studies have suggested that subchondral bone structure is altered in patients with osteoarthritis (OA). Mansell et al, for example, evaluated the concentration of proactive and active matrix metalloproteinase 2 and that of alkaline phosphatase in subchondral bone specimens obtained from femoral heads collected from women undergoing total hip replacement surgery for osteoporosis or OA and from normal autopsy subjects (1). They showed that subchondral bone turnover was significantly higher in patients with OA than in both patients with osteoporosis and controls (1). Dieppe et al showed that increased subchondral bone turnover, as suggested by an increased bone scintigraphic signal at the affected knee, was predictive of OA progression in the following 5 years (2). They reported similar results for OA of the hand (3). More recently, the same group of investigators showed that in the osteoarthritic knee,

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scintigraphic abnormalities correlated with synovial fluid levels of osteocalcin, a marker of bone formation (4). The results of these studies suggest that a measurement of bone turnover in patients with OA could be used to identify those whose disease is likely to progress. However, neither bone scintigraphy nor measurement of synovial fluid concentrations of biochemical markers would be practical for widespread use, given the invasivity of both methods and the radiation load to which the patient would be exposed during bone scintigraphic examinations.

In recent years, different biochemical markers of bone turnover have been developed and validated both in humans and in animal models (5). These markers are secreted during bone formation or following bone resorption and can be measured in serum and/or urine. The most extensively studied and validated markers are osteocalcin and bone alkaline phosphatase (for bone formation) and deoxypyridinoline and type I collagen C-terminal and N-terminal telopeptides (CTx and NTx, respectively) (for bone resorption). Different studies have shown that biochemical markers of bone turnover correlate with bone turnover levels as measured by histomorphometry or calcium kinetics. Biochemical markers of bone are very sensitive to changes in bone turnover; however, because they are derived from the whole skeleton, information on particular skeletal sites can only be inferred.

Previous studies have examined the level of markers of bone resorption and formation at different sites in patients with OA (6–19). The majority of these studies showed an increase in bone markers in patients with OA (6–9,11–17), although some studies demonstrated either no change or even decreases in bone turnover in OA (10,18,19). A possible explanation for these discrepancies may reside in the relative study designs. These studies have been cross-sectional, often included patients with both progressive and nonprogressive knee OA, and, in most cases, the patient population included both men and pre- and postmenopausal women of a wide age range.

In an effort to overcome some of these limitations, we conducted tests to determine whether markers of bone turnover are increased in patients with OA, and whether there is a correlation between changes in bone turnover and progression of OA. Urinary excretion of 2 markers of bone resorption (NTx and CTx) was measured in samples collected from a subset of postmenopausal women participating in the Chingford study, characterized for the presence of progressive knee OA.

## PATIENTS AND METHODS

**Subjects.** The study subjects were from the Chingford Study population. This is a well-documented cohort of 1,003 women, ages 45 to 64 years, for whom baseline observations were made in 1988–1989 (20). The cohort, which was predominantly white, was seen annually. At baseline, all subjects underwent anteroposterior weight-bearing radiography of the knee, taken in full extension. In addition, bone mineral density (BMD) of the lumbar spine was measured. Radiographs of the knee were repeated 4 years later. Blood and urine samples were collected at baseline and annually thereafter and stored at  $-45^{\circ}\text{C}$  in aliquots, without freeze-thawing, until the time of analysis. At both baseline and 4 years later, all subjects completed a standardized questionnaire on medical history. Height, weight, and use of concomitant medications were recorded, and women receiving bone-modifying drugs (e.g., estrogen replacement therapy) were excluded.

Based on a diagnosis of either knee OA or osteoporosis at baseline and on radiologic evidence of progression of knee OA 4 years later, postmenopausal women (defined as  $>12$  months after the last menses or ovariectomy) from the original group were selected to populate the 4 groups in this study. The control group ( $n = 50$ ) comprised all postmenopausal women who had normal knee radiographs at baseline and 4 years later and no clinical sign of osteoporosis, defined as no clinical osteoporotic fractures and a T score at the spine that was higher than the World Health Organization threshold T score of less than  $-2.5$ . The progressive OA group ( $n = 71$ ) comprised all postmenopausal women who either had no sign of knee OA at baseline and a diagnosis of knee OA 4 years later or were diagnosed as having knee OA at baseline, with worsening of OA 4 years later. Women in this group had no clinical signs of osteoporosis. At baseline, 45% had radiographic joint space narrowing (JSN), and 41% had osteophytes. The nonprogressive OA group ( $n = 36$ ) comprised all postmenopausal women who were diagnosed as having knee OA at baseline and whose OA had not progressed 4 years later. All of these women had no clinical signs of osteoporosis. At baseline, 39% had radiographic JSN, and 97% had osteophytes. The osteoporosis group ( $n = 59$ ) comprised all postmenopausal women who had osteoporosis and normal knee radiographs both at baseline and 4 years later.

**Definition of OA and osteoporosis.** Knee radiographs were taken with subjects in the weight-bearing, fully extended position. Films were read by a trained examiner for the presence of knee osteophytes and JSN, using a 0–3 scale of severity. Knee OA was defined as grade  $\geq 1$  osteophytes or JSN. Progressive OA was defined as a change of  $\geq 1$  osteophytes or JSN from grade 1 at baseline (21). Baseline groups were compared using a composite radiography scale, which was calculated by adding all possible individual scores for JSN and osteophytes. Lumbar spine (L1–L4) and total hip BMD were measured using Hologic QDR 1000 dual x-ray absorptiometry (Bedford, MA). For this study, osteoporosis was defined as an age-dependent WHO-defined T score of less than  $-2.5$  and an age-independent Z score less than  $-1$  for the lumbar spine.

**Analysis of markers of bone turnover.** Fasting urine samples (second morning void) collected from each patient at baseline, year 1, and year 2 were analyzed. The level of NTx

**Table 1.** Characteristics of study groups at baseline\*

Parameter	Study group			
	Control	Progressive osteoarthritis	Nonprogressive osteoarthritis	Osteoporosis
Age, years	55.9 ± 0.77	58.8 ± 0.57	57.4 ± 0.79	58.4 ± 0.59
Years since menopause	8.3 ± 0.75	10.4 ± 0.71	8.4 ± 0.94	10.7 ± 0.83
BMI, kg/m <sup>2</sup>	26.3 ± 0.71	27.1 ± 0.57	26.6 ± 0.61	23.6 ± 0.32
BMD, hip, gm/cm <sup>2</sup>	0.84 ± 0.017	0.76 ± 0.014	0.80 ± 0.021	0.63 ± 0.017
BMD, lumbar spine, gm/cm <sup>2</sup>	1.15 ± 0.014	0.97 ± 0.018	1.03 ± 0.028	0.70 ± 0.007
T score, lumbar spine	0.9 ± 0.12	-0.7 ± 0.16	-0.2 ± 0.25	-3.1 ± 0.06

\* Values are the mean ± SEM. BMI = body mass index; BMD = bone mineral density.

was measured using a commercial kit with a monoclonal antibody specific for the crosslinked type I collagen N-terminal telopeptide (Osteomark; Ostex, Seattle, WA). The level of CTx was measured using a commercial enzyme-linked immunosorbent assay (Crosslaps; Osteometer Biotech, Herlev, Denmark) with a polyclonal antibody raised against a synthetic sequence of type I collagen C-terminal telopeptide. Creatinine was measured by an automated assay based on the Jaffe method. Urinary excretion of NTx and CTx was expressed as a ratio with creatinine excretion: NTx = nmoles of bone collagen equivalent:mmoles of creatinine; CTx = mg:mole of creatinine).

**Statistical analysis.** Descriptive statistics are presented for each variable. Urinary excretion of NTx and CTx was compared among groups either as the by-patient average of the 3 available time points (the composite score) or separately for each time point. In both situations, values were analyzed by pairwise comparisons, using analysis of variance (ANOVA) of ranked response in order to satisfy the ANOVA assumptions for the analyses without covariate adjustment and for the analyses after adjusting for age, body mass index (BMI), BMD, and past use of hormone replacement therapy (HRT). The ANOVA without covariate adjustment is equivalent to the Kruskal-Wallis nonparametric test. The area under the curve (AUC) of marker excretion by time plots was also examined. The 5% significance level was used for all tests, and all tests were 2-sided. Groups and hypotheses about the groups were determined a priori. *P* values were not corrected for multiple comparisons, because the analyses were exploratory.

## RESULTS

Table 1 shows the baseline characteristics of the subjects recruited into this study. Age and years since menopause were similar among the groups at baseline. The mean ± SD baseline composite radiographic OA score for women with progressive OA was 2.17 ± 2.99, which was similar to that for women with nonprogressive OA (2.33 ± 1.55). At year 5, the mean ± SD score for subjects in the progressive OA group had increased to 3.77 ± 3.46. As expected, women with osteoporosis had the lowest BMI (10% lower than in controls), BMD in the lumbar spine (39% lower than in controls), and T

scores (-3.1 versus 0.9 in the control group); BMD in the hip was 25% lower in women with osteoporosis than in controls.

As shown in Table 2, the average (3 time points) NTx and CTx urinary excretion was higher in patients with progressive knee OA and in patients with osteoporosis than in controls (*P* < 0.001). Urinary excretion of NTx was significantly higher in women with progressive OA than in those with nonprogressive OA (*P* < 0.01). Urinary excretion of NTx and CTx was higher in women with osteoporosis than in those with nonprogressive OA (*P* < 0.01). In contrast, urinary excretion of NTx and CTx did not differ significantly between women with osteoporosis and those with progressive OA.

As shown in Figure 1, at all time points, the level of urinary excretion of both NTx and CTx was significantly higher in women with progressive knee OA than in controls (*P* < 0.05). At all time points, urinary excretion of NTx and CTx was similar in patients with nonprogressive OA and controls. Both at baseline and at

**Table 2.** Average urinary excretion of type I collagen N-terminal and C-terminal telopeptides\*

	Type I collagen telopeptides	
	N-terminal	C-terminal
Group 1	37.0 (25.5, 60.4)	126.5 (77.5, 205.8)
Group 2	53.9 (39.6, 79.1)†	214.0 (145.2, 285.4)‡
Group 3	40.1 (28.1, 56.5)§	180.6 (96.1, 246.7)¶
Group 4	59.8 (44.1, 80.4)†	236.5 (178.9, 293.2)†

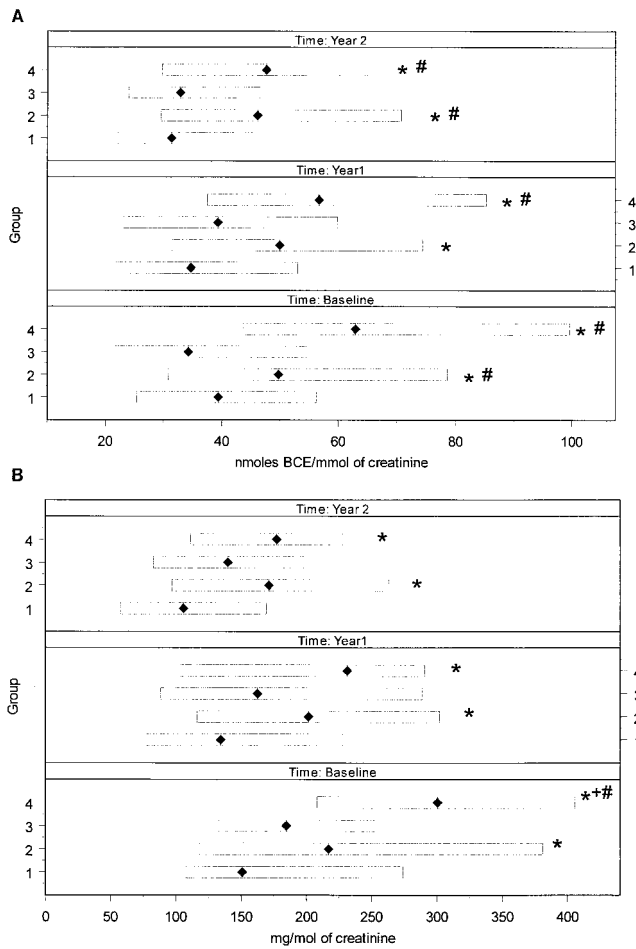
\* Values are the unadjusted medians (lower interquartile, upper interquartile) of measurements from baseline, year 1, and year 2. Group 1 = control; group 2 = progressive knee osteoarthritis (OA); group 3 = nonprogressive knee OA; group 4 = osteoporosis; N-terminal = nmoles bone collagen equivalent:mmoles creatinine; C-terminal = mg:mole creatinine.

† Significantly different from groups 1 and 3.

‡ Significantly different from group 1.

§ Significantly different from groups 2 and 4.

¶ Significantly different from group 4.



**Figure 1.** Urinary excretion of type I collagen N-terminal telopeptides (A) and type I collagen C-terminal telopeptides (B) at 3 different time points. Group 1 = control; group 2 = progressive knee osteoarthritis (OA); group 3 = nonprogressive knee OA; group 4 = osteoporosis. Bars show the lower and upper interquartiles; diamonds within the bars show the median. \* = significantly different from group 1; + = significantly different from group 2; # = significantly different from group 3; BCE = bone collagen equivalent.

year 2, the level of urinary excretion of NTx was significantly higher in women with progressive knee OA compared with those with nonprogressive knee OA. Finally, urinary excretion of NTx and CTx was not significantly different between women with progressive knee OA and those with osteoporosis at any time point, except at baseline ( $P < 0.01$ ) for CTx. The significance of the differences in the level of urinary excretion of NTx and CTx between women with progressive OA and either those with nonprogressive OA or controls did not markedly change after adjusting for age, baseline BMI and BMD, and past use of HRT.

When the data were also analyzed by AUC, the

results were unchanged. No clear or consistent temporal trends were seen when individual time points in relation to the timing of OA progression were evaluated. When only subjects who had osteophytes at baseline were compared, the 3-year median levels of NTx were 37.1 for the group with progressive OA and 33.2 for the group with nonprogressive OA; the median levels of CTx were 159.1 for the group with progressive OA and 143.8 for the group with nonprogressive OA.

After stratifying for changes in specific OA features, there was no clear difference between the association of markers and progression of OA based on osteophyte change only and JSN change only. Although no results reached significance, the trends were the same as those for the composite score: for osteophytes ( $n = 59$ ), the median level of NTx was 43.8 in patients with progressive OA versus 40.4 in those with nonprogressive OA; for JSN ( $n = 42$ ), the median levels were 63.7 and 40.4, respectively. For osteophytes, the median levels of CTx were 167.5 in patients with progressive OA versus 181.8 in those with nonprogressive OA; for JSN, the median levels were 221.5 and 198.2, respectively. We were therefore unable to clearly equate the changes in resorption with specific features, though the differences for JSN were more marked.

An additional subanalysis demonstrated no consistent differences when incident cases of progressive OA ( $n = 37$ ) were separated from cases of prevalent progressive OA ( $n = 34$ ), although composite resorption levels for the former group tended to be higher (NTx, 54.3 versus 46.8; CTx, 246 versus 174). The number of cases of bilateral progression ( $n = 3$ ) was insufficient to allow analysis of the effects of disease severity on marker levels.

## DISCUSSION

This study is the first to show that postmenopausal women with progressive knee OA have increased bone resorption, as demonstrated by increased urinary excretion of NTx and CTx, both of which are validated markers of bone resorption. Such an increase was not observed in postmenopausal women with stable (nonprogressive) knee OA. The increase in urinary excretion of NTx and CTx in postmenopausal women with progressive knee OA was similar to that detected in age-matched women with osteoporosis.

Previous studies have examined the level of markers of bone resorption and bone formation at different sites in patients with OA. In particular, urinary excretion of pyridinium crosslinks (6–15) and CTx (8,10)

and blood levels of bone sialoprotein (BSP) (16,17) have been measured to evaluate bone resorption, and osteocalcin (4,18,19) concentrations, in both blood and synovial fluid, have been measured to evaluate bone formation. Not all studies have shown concordant results, although the majority of them have demonstrated an increase in bone markers in patients with knee OA (6–9,14,15,17). Some studies have also shown a correlation between the level of bone markers and OA severity (8,15). However, all of these studies were cross-sectional, none of them compared the level of bone markers in patients with progressive OA and those with nonprogressive knee OA, and, in most cases, the patient population included both men and women. In addition, often both premenopausal and postmenopausal women were included, or no information was given regarding the menopausal status of the female patient population.

None of the bone markers that have been studied thus far and are currently available are specific for subchondral bone, and, because all are circulating markers, none of them is specific for a certain skeletal site (e.g., the knee). Even BSP, which is enriched in subchondral bone (22), is present throughout the skeleton. Levels of bone markers can vary between men and women and can also vary among women depending on ovarian status (5). For all of the markers tested to date, a 50–100% increase is seen after onset of menopause. For this reason, it is very important that the patient groups compared are matched by sex and menopausal status. In the present study, all 4 groups comprised postmenopausal women, and the groups were similar in terms of age and time since onset of menopause. The differences in the other baseline characteristics, such as BMD, were not unexpected, because of the group selection criteria.

Although NTx and CTx have been shown to be very sensitive to changes in bone resorption (23), they also have a high degree of variability (24). Because of such variability, we averaged the results obtained from 3 time points ~1 year apart. During the time of the study, none of the patients received long-term treatment with compounds known to alter bone metabolism. Therefore, the baseline characteristics that were used to separate the patients into 4 different groups were not expected to change to any extent throughout the 4 years of the study. We also analyzed the single time points separately to evaluate whether different patterns could be distinguished among the different study groups.

The median levels of urinary excretion of NTx and CTx clearly indicate increased bone resorption in women with progressive knee OA compared with both

healthy controls and women with nonprogressive knee OA. These results were significant for both NTx and CTx, with the only exception being CTx in women with progressive OA versus those with nonprogressive OA. Urinary excretion of NTx and CTx did not differ between women with progressive knee OA and those with osteoporosis, suggesting a comparable level of bone resorption in these 2 groups of patients.

The analysis of the single time points did not show a different pattern, even though all of the individual differences did not reach statistical significance. At the last time point, urinary excretion of NTx and CTx was reduced in all groups. We thoroughly analyzed whether there were any reasons for a systematic difference (e.g., changes in treatment, new diseases, technical issues in either sample collection or sample storage), and nothing could explain such changes. However, the pattern of results obtained at the last time point did not differ from those obtained using the averaged results for NTx and CTx or those obtained at the other time points. The analysis of the single time points did not show any phased change in the level of bone resorption, but this may be attributable to the relatively short period of time between collection of the first and last samples.

This study has several limitations that merit discussion. First, the study was conducted in a population of relatively young postmenopausal women. The reason for selecting a relatively homogeneous patient population has been addressed above. Although such selection criteria have rendered our results more robust, the results may not be automatically reapplied to OA patients who are male or premenopausal. Similarly, our patients had relatively moderate knee OA; therefore, the results cannot be extrapolated to more severe, bilateral, or end-stage cases. Indeed, some of the differences in the results published in the literature may be explained by the different ages or level of OA severity in the patients analyzed.

The definition of OA progression was based on changes in radiographs according to a well-validated scoring system (21). However, the radiographs were taken with patients in a fully extended, weight-bearing position, which recently has been shown to be less sensitive than the semiflexed weight-bearing position for the evaluation of JSN (25). For this reason, we might have miscoded patients for whom changes on the radiographs were too subtle to detect with this method as having nonprogressive OA. Such misclassification would have influenced the study toward a null result. The subanalyses we performed could not separate the effects of osteophytes or incident versus prevalent progressive

disease. The lack of a consistent significant association may be attributable to the small number of patients in these subgroups. A much larger study would be needed to do this accurately. In reality, it is likely that radiologic incidence and progression are similar pathologically but may reflect different stages.

We recently demonstrated in the Chingford study that the majority of subjects with minor or doubtful knee osteophytes (Kellgren/Lawrence grade 1, which, in most studies, would be counted as normal) progressed over 5 or 10 years to develop Kellgren/Lawrence grades 2–3 (26). We therefore believe that any distinction between radiologic incidence and progression as defined in these studies is somewhat arbitrary, depending on the sensitivity of grading scores. Caution should also be exercised when comparing progression of different features, because a recent study demonstrated that a method of scoring progression of knee OA based on JSN alone performed poorly (27). Furthermore, the difference in baseline osteophytes between patients with progressive OA and those with nonprogressive OA may reflect different phases of the disease process rather than purely individual differences.

In the current study, we measured only markers of bone resorption. A coupling between bone resorption and bone formation normally exists in all patients, although these processes may not be properly balanced, as occurs after menopause with an increased rate of resorption relative to the rate of formation. Only occasionally do the 2 processes work in different directions. For example, the onset of treatment with compounds that inhibit bone turnover is followed by a rapid decrease in bone resorption that, only a few weeks later, is followed by a similar change in bone formation (23). No study yet exists showing an uncoupling between bone resorption and bone formation in patients with OA. However, it is worth mentioning that 2 of the 3 studies that showed a decrease in bone markers measured only osteocalcin, a validated marker of bone formation (18,19). Future studies are required to evaluate whether bone resorption and formation are uncoupled in OA.

As discussed above, our study was designed to control at least some confounding factors, the most important of which were menopause and age. BMI and BMD were similar among controls and women with OA but were significantly (and as expected) reduced in women with osteoporosis. In older populations (age >65 years), spine BMD measurements can be artefactually increased by a few percentage points in patients with OA of the spine (28). Our results, however, were similar to those for hip BMD, and it is unlikely that many patients

with true osteoporosis were incorrectly classified as having OA using this selection method in this age group. Also, to our knowledge, patients were not taking any medication during the study period from 1988 to 1993, which might have biased the results. Knee pain and inactivity were unlikely to be confounders in this study, because there was no correlation between pain reporting and physical activity levels in the women. Despite having controlled for many of these confounding factors during patient selection, we also tested whether including age, BMI, BMD, and past use of HRT as covariates in the analysis would alter the results. The time point results between women with progressive OA and either those with nonprogressive OA or controls did not change substantially when this was done, supporting the robustness of our data.

In conclusion, our data show that bone resorption is increased in patients with progressive knee OA and not in those whose knee OA does not progress. The increase in bone resorption seen in patients with progressive knee OA is similar to that seen in patients with osteoporosis. The question of whether the problem is contributory or merely a secondary phenomenon cannot be answered by this study, nor could we distinguish the relative roles of osteophytes and JSN. These findings should, however, stimulate further work to determine the clinical usefulness of bone markers as prognostic indicators of OA progression, or the role of therapeutic modulation of bone turnover in reducing progression of OA.

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