

Relationship Between Bone Markers and Knee Cartilage Volume in Healthy Men

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ABSTRACT. *Objective.* To determine the relationship between biochemical bone markers and knee cartilage volume and cartilage loss over 2 years; and to investigate whether bone markers are useful to predict the cartilage loss in healthy men.

Methods. Forty healthy Caucasian men (mean age 52.3 yrs) with no symptoms of osteoarthritis (OA) were recruited. Each subject had magnetic resonance imaging (MRI) performed on his dominant knee at baseline and 2 years later. Serum level of osteocalcin (OC), urinary levels of pyridinoline (PYD) and deoxypyridinoline (DPD), and total body bone mineral content (BMC) were measured at baseline. Tibial plateau bone size was measured at baseline. Tibial cartilage volume was measured at baseline and at followup, by means of image processing.

Results. Twenty-eight men (70%) completed the longitudinal MRI component of the study. At baseline, no significant associations were observed between values of serum OC or urine PYD and DPD and tibial cartilage volume. Higher baseline serum OC level tended to be associated with a decreased rate of cartilage loss ($p = 0.06$); no significant association was shown between baseline urine PYD and DPD and tibial cartilage loss, after adjusting for age, body mass index, total body BMC, and tibial plateau bone size.

Conclusion. Higher baseline serum OC level tended to be associated with a decreased rate of cartilage loss, suggesting that increased bone formation may protect against tibial cartilage loss over 2 years. Studies are needed to determine the role of bone metabolism in the pathogenesis of knee OA. (J Rheumatol 2005;32:2200–4)

Key Indexing Terms:

OSTEOCALCIN
CARTILAGE VOLUME

PYRIDINOLINE
BONE MARKERS

DEOXYPYRIDINOLINE
OSTEOARTHRITIS

A number of serum and urinary biochemical markers have been validated for assessing the rate of bone turnover¹. For bone formation, one of the most specific markers is osteocalcin (OC). The most sensitive resorption markers are collagen crosslinks, including pyridinoline (PYD) and deoxypyridinoline (DPD).

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An increase in serum or urine concentrations of OC, PYD, and DPD has been reported in patients with osteoarthritis (OA) compared with controls^{2–9}, suggesting an increased rate of bone turnover in patients with OA. Most of these data were obtained from cross-sectional studies. Few studies have assessed the relationship between structural change of OA and biochemical markers. Moreover, structural severity was generally measured by change in radiological grade or joint space width. One longitudinal study showed that the one-year increase in OC was significantly associated with loss of joint space width in subjects with knee OA over 3 years¹⁰. The longitudinal Michigan Bone Health Study showed serum OC level was lower in 3-year incident cases of hand or knee OA compared with women without OA¹¹. There are no data available on serum or urine OC, PYD, and DPD in the early, pre-OA state.

Radiography of the knee provides an indirect assessment of joint cartilage. Recently, magnetic resonance imaging (MRI) has been shown to be a valid, safe, noninvasive, sensitive, and reproducible technique for measuring knee cartilage thickness¹² and volume^{12–14} *in vivo*. Knee cartilage volume is also a useful method for examining healthy subjects, before the onset of knee OA^{15–17}.

We conducted a prospective cohort study to determine the relationship between bone markers and knee cartilage volume and cartilage loss over 2 years in healthy men with no

symptoms of knee OA, to investigate whether bone markers are useful to predict the cartilage loss in healthy men.

MATERIALS AND METHODS

Forty healthy, Caucasian men aged 24–75 years with no symptoms of knee OA were recruited through advertising in newspapers, through sporting clubs, and through the hospital staff association. Exclusion criteria included history of OA or osteoporosis, previous significant knee injury requiring non-weight-bearing treatment for > 24 h or surgery (including arthroscopy), contraindication to MRI including pacemaker, metal sutures, presence of shrapnel, iron filings in the eye. The study was approved by the Human Research Ethics Committee of the Royal Melbourne Hospital, Victoria, Australia.

Weight was measured to the nearest 0.1 kg (shoes and bulky clothing removed) using a single pair of electronic scales. Height was measured to the nearest 0.1 cm (shoes removed) using a stadiometer. Body mass index (BMI; weight/height², kg/m²) was calculated. Current total physical activity was a composite score of total amount of walking (0–4) + activity at home (0–4) + sporting activity (0–4)¹⁸. Each subject had an MRI performed on his dominant knee, defined as the lower limb from which they step off when walking, at baseline and about 2 years later.

Knees were imaged in the sagittal plane on a 1.5-Tesla whole-body MR unit (Signa Advantage Echospeed, GE Medical Systems, Milwaukee, WI, USA) using a commercial transmit-receive extremity coil, with sequence and parameters as described^{15,17,19}. Knee cartilage volume was measured by 2 independent observers by means of image processing on an independent work station using the software program Osiris as described^{15,17,19}. The coefficients of variation (CV) for total, medial, and lateral tibial cartilage volume measures were 2.6%, 3.3%, and 2.0%, respectively¹⁷. Medial and lateral tibial plateau areas were determined by creating an isotropic volume from the input images. This was reformatted in the axial plane. The areas were directly measured from these images. CV were 2.3% for medial and 2.4% for lateral tibial plateau areas¹⁷. MRI scans were examined for features of OA as described²⁰.

Early morning blood samples and first-void urine samples were collected from all subjects within 8 weeks of their MRI at baseline. Serum and urine specimens were stored at –70°C before measurement. Total urine PYD corrected for creatinine was measured after column chromatography using high performance liquid chromatography (HPLC). The intra- and inter-assay CV were 16% and 18.7%, respectively. Total urine DPD corrected for creatinine was also measured using HPLC. The intra- and inter-assay CV were 11.6% and 13%, respectively. Serum OC was measured using a 2-site immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX, USA) in a single assay. The intra- and inter-assay CV were 3.1% and 5.3%, respectively. Total body bone mineral content (TBBMC) was measured by dual energy x-ray absorptiometry (DEXA) using a Hologic QDR 1000 W densitometer. The CV for TBBMC was 0.6%. All laboratory results were analyzed blind to the clinical data.

Descriptive statistics for characteristics of the subjects were tabulated. Linear regression was used to examine the relationship between bone markers and knee cartilage volume at baseline, and the relationship between bone markers and cartilage loss over 2 years. Annual tibial cartilage loss was calculated as (baseline cartilage volume – followup cartilage volume)/(time between MRI scans), and also expressed as percentage loss. Initial tibial cartilage volume and tibial cartilage loss were regressed against bone markers in univariate regression analyses, and in multivariate regression models adjusting for potential confounders including age, BMI, tibial bone size, and total body BMC. A p value less than 0.05 (2-tailed) was considered statistically significant. All analyses were performed using the SPSS statistical package (standard version 11.5.0; SPSS, Chicago, IL, USA).

RESULTS

Baseline characteristics of the study participants are presented in Table 1. Twenty-eight men (70%) were followed

by a repeated MRI on the same knee in 2.7 ± 0.2 years (range 2.5–3.0 yrs). The following were reasons for loss to followup: one died, 3 were overseas, 3 refused to participate, and 5 were not contactable. When the noncompleted subjects are compared to completed subjects for the following characteristics, no significant differences were observed: age (p = 0.79), BMI (p = 0.36), baseline tibial cartilage volume (p = 0.91), TBBMC (p = 0.15), physical activity (p = 0.33), OC (p = 0.19), PYD (p = 0.79), and DPD (p = 0.83); the exception was greater baseline tibial plateau bone size (p = 0.02).

At baseline, no significant associations were observed between values of serum OC or urine PYD and DPD and tibial cartilage volume in univariate analysis, and also in multivariate analysis adjusting for age, BMI, TBBMC, and tibial plateau bone size (Table 2). Adjustment for physical activity did not alter the results (data not shown). When medial and lateral tibial cartilage volumes were analyzed separately, similar results were observed (data not shown).

In univariate analysis, baseline bone markers studied were not associated with percentage tibial cartilage loss. In multivariate analysis, higher baseline serum OC level tended to be associated with a decreased rate of tibial cartilage loss (p = 0.06); no significant association was shown between baseline urine PYD and DPD and tibial cartilage loss, after adjusting for age, BMI, TBBMC, and tibial plateau bone size (Table 3). Adjustment for physical activity did not alter the results (data not shown). When medial and lateral tibial cartilage losses were analyzed separately, similar results were observed (data not shown).

Table 1. Characteristics of participants.

	Mean (SD) (N = 40), Range
Age, yrs	52.3 (13.0) 24.0–75.0
Weight, kg	79.7 (12.8) 56.0–119.0
Height, cm	176.7 (6.7) 165.0–193.7
BMI, kg/m ²	25.5 (3.6) 19.4–36.7
Time between MR scans, yrs	2.7 (0.2) 2.5–3.0
Physical activity score	6.5 (1.9) 3.0–11.0
Total body bone mineral content, units	2851.0 (420.6) 1725.0–4096.0
Tibial cartilage volume, μl	5693.1 (1016.8) 3189.0–7997.0
Tibial plateau bone size, mm ²	3341.2 (566.7) 2221.0–4650.0
Pyridinoline/Cr, nmol/mmol Cr	50.7 (25.8) 6.4–123.3
Deoxypyridinoline/Cr, nmol/mmol Cr	8.6 (4.3) 3.0–20.3
Osteocalcin, ng/ml	4.5 (2.0) 1.3–8.3

Table 2. Relationship between bone markers and tibial cartilage volume (μl).

	Univariate Analysis, Regression Coefficient (95% CI)	p	Multivariate Analysis*, Regression Coefficient (95% CI)	p
Osteocalcin**	-136.18 (-301.15, 28.80)	0.10	-119.10 (-297.38, 59.19)	0.18
Pyridinoline†	2.64 (-11.42, 16.70)	0.70	4.66 (-9.73, 19.05)	0.51
Deoxypyridinoline††	-13.89 (-98.93, 71.15)	0.74	3.39 (-87.49, 94.27)	0.94

* Multivariate analysis with age, BMI, tibial plateau bone size, and total body BMC in regression equation. ** Change in tibial cartilage volume (μl) for every 1 ng/ml increase in osteocalcin. † Change in tibial cartilage volume (μl) for every 1 nmol/mmol Cr increase in pyridinoline. †† Change in tibial cartilage volume (μl) for every 1 nmol/mmol Cr increase in deoxypyridinoline.

Table 3. Relationship between bone markers and annual tibial cartilage loss (%) over 2 years.

	Univariate Analysis, Regression Coefficient (95% CI)	p	Multivariate Analysis*, Regression Coefficient (95% CI)	p
Osteocalcin**	-0.19 (-0.48, 0.10)	0.19	-0.35 (-0.72, 0.02)	0.06
Pyridinoline†	0.01 (-0.01, 0.03)	0.29	0.01 (-0.01, 0.03)	0.40
Deoxypyridinoline††	0.06 (-0.07, 0.20)	0.33	0.04 (-0.12, 0.20)	0.58

* Multivariate analysis with age, BMI, tibial plateau bone size, and total body BMC in regression equation. ** Change in tibial cartilage loss (%) for every 1 ng/ml increase in osteocalcin. † Change in tibial cartilage loss (%) for every 1 nmol/mmol Cr increase in pyridinoline. †† Change in tibial cartilage loss (%) for every 1 nmol/mmol Cr increase in deoxypyridinoline.

DISCUSSION

In this prospective cohort study, we found higher baseline serum OC level tended to be associated with a decreased rate of cartilage loss in healthy men over 2 years, after adjustment for potential confounders including age, BMI, physical activity, total body bone mineral content, and tibial plateau bone size. No significant association was shown between serum OC or urine PYD and DPD levels and baseline tibial cartilage volume, independent of age, BMI, TBBMC, and tibial plateau bone size.

Markers of bone formation and resorption have been compared between subjects with OA and healthy controls. Garnero, *et al* reviewed several cross-sectional studies that found elevated levels of biochemical markers of bone, cartilage, and synovium in OA²¹. The serum OC concentration and urinary excretion of pyridinium crosslinks have been shown to be increased in patients with OA compared to controls, suggesting an increased rate of bone turnover in this condition²⁻⁹. Recent studies have shown a correlation between pyridinium crosslinks and radiographic changes in OA^{7,22,23}. Urine pyridinium crosslinks increased in the late stages of OA (grade 3 and 4, with subchondral bone changes), suggesting that in OA these markers may mainly reflect the degradation of the bone^{7,22,23}. In contrast, some studies have shown lower bone marker levels suggesting a systemic decrease in bone turnover^{11,24,25} or no significant difference^{22,26,27} in OA compared to controls. However, none of these studies allowed for the fact that there may be increased skeletal mass to account for the increase in crosslink excretion. Peel, *et al* found urinary excretion of

DPD was lower in women aged 50 to 85 years with spinal OA compared with women without the disease²⁵. Unlike previous studies, bone mass was taken into account in this study. We observed no significant associations between values of serum OC or urine PYD and DPD and tibial cartilage volume at baseline in healthy men. We also adjusted for total bone mass. Our study differed from the previous studies, which were performed in subjects with knee OA.

Few studies have examined the association between bone markers and progression of joint destruction. Bruyere, *et al* found that baseline biochemical markers were not correlated with 3-year percentage changes in joint space width¹⁰. However, they showed that the one-year increase in OC was significantly correlated with 3-year progression in joint space narrowing in knee OA. In contrast, we found that higher baseline serum OC level tended to be associated with a decreased rate of cartilage loss. Bone turnover has been assessed by measuring serum OC (bone formation) and urinary pyridinium crosslinks (bone resorption). Osteocalcin or bone Gla protein, a vitamin K-dependent bone protein, is unique to bone, accounting for up to 20% of the noncollagenous proteins in adult bone²⁸. Synthesized by mature osteoblasts, it has been described as a sensitive and specific marker of osteoblastic activity²⁹. The OC found in serum comes from new cellular synthesis rather than from the release of bone matrix protein during bone resorption³⁰. The serum level of intact OC reflects the rate of bone formation^{31,32}. Our data in healthy men suggest that increased bone formation (as measured by serum OC) protects against cartilage loss in healthy subjects. It may be that once OA is

established the increased bone turnover has a negative effect, as seen in the study by Bruyere, *et al*¹⁰.

A limitation of our study is that we included only men. Whether our findings are generalizable to women will need to be determined. The study was potentially limited by a relatively small sample size. However, the study demonstrated sufficient power to detect a significant relationship between knee cartilage volume and serum OC in the longitudinal analyses. The strength of this study is that we used a novel method for assessing knee cartilage volume in healthy asymptomatic subjects. Recent studies have shown that quantitative assessment of knee cartilage volume is reliable and reproducible, being associated with OA risk factors such as sex, age, BMI, and use of hormone replacement therapy¹⁵⁻¹⁷. Cartilage volume measured by MRI is significantly correlated with radiographic features of the knee OA, including joint space narrowing³³, which is a commonly employed surrogate measure of articular cartilage. By the time the first changes of radiological OA are detected, 13% of knee cartilage has already been lost³⁴. In a longitudinal study we have shown that subjects with knee OA lose 5% of their tibial cartilage per year³⁵ and that loss of tibial cartilage correlates with worsening of symptoms³⁶ and predicts knee replacement³⁷. The emerging evidence suggests that there is a continuum from a normal knee to an osteoarthritic knee. This is supported by a recent study that showed that quantitative analysis of OA by MRI is feasible using T and Z scores³⁸. A major strength of using cartilage volume is that we can examine the state of knee cartilage as a continuous variable from the normal to the prediseased to the early diseased state. We measured only tibial cartilage volume in these subjects, having previously shown that tibial and femoral cartilage volumes³⁹ and change in tibial and femoral cartilage⁴⁰ are correlated.

Although no significant associations were observed between values of serum OC or urine PYD and DPD and tibial cartilage volume, higher baseline serum OC level tended to be associated with a decreased rate of cartilage loss in healthy men. Increased bone formation may protect against tibial cartilage loss over 2 years. Further studies will be needed to understand the role of bone metabolism in the pathogenesis of knee OA.

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