

EXTENDED REPORT

Factors influencing longitudinal change in knee cartilage volume measured from magnetic resonance imaging in healthy men

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Objective: To determine whether the amount of joint cartilage in healthy, middle aged men is stable or changes over time, and what factors may influence this.

Methods: In a cohort study, 28 healthy men (70% of the original cohort; mean (SD) age, 51.9 (12.8) years) had baseline knee magnetic resonance imaging (MRI) of their dominant knee and repeat MRI of the same knee approximately 2.0 years later. Knee cartilage volume was measured at baseline and follow up. Risk factors assessed at baseline, including sex hormones and metabolic bone markers, were tested for their association with change in knee cartilage volume over time.

Results: Mean (SD) reduction in tibial cartilage volume per year was 162 (93) μ l. This represented a 2.8% reduction in total tibial articular cartilage per year (95% confidence interval, 0.2% to 5.5%). Tibial cartilage loss was associated with serum free testosterone level, independently of age, body mass index, baseline tibial cartilage volume tibial plateau area, and total bone mineral content. Overall, testosterone accounted for 14.5% (partial r^2) of the variation in change in tibial cartilage volume. There was a trend towards a positive association between tibial cartilage loss and urinary N-telopeptide cross-links of type I collagen (Ntx) ($p=0.057$).

Conclusions: Further studies will be required to determine whether hormonal manipulation or treatment with antiresorptive drugs will reduce the risk of knee osteoarthritis in men in later life.

Osteoarthritis is a disease of joints involving both cartilage and bone in the disease process. With increasing disease severity, articular cartilage is lost. However, although much is known about what happens at the level of the joint after disease onset, little is known about initiating factors or about the pre-disease state in healthy humans. Factors influencing the incidence of osteoarthritis have been identified through epidemiological and small group studies. These factors include sex (women, especially after entering the menopause),¹ obesity,² inheritance,³ knee injury,⁴ and quadriceps strength.⁵ Understanding factors that influence joint cartilage in health and disease is important in the prevention and management of osteoarthritis.

Until recently, few data were available on factors affecting cartilage in healthy subjects owing to the limitations in assessing joint cartilage non-invasively. There has been recent interest in examining joint cartilage using magnetic resonance imaging (MRI). MRI allows visualisation of all components of the joint simultaneously, and is a valid and reproducible method for examining articular cartilage volume.^{6,7} It has also been shown to be sensitive to change in longitudinal studies in both people with osteoarthritis⁸ and healthy subjects.⁹ Cartilage volume measured by MRI is significantly correlated with radiographic features of knee osteoarthritis, including joint space narrowing,¹⁰ which is a commonly employed surrogate measure of articular cartilage. By the time the first changes of radiological osteoarthritis are detected, 13% of knee cartilage has already been lost.¹¹ In a longitudinal study we have shown that subjects with knee osteoarthritis lose 5% of their tibial cartilage per year⁸ and that loss of tibial cartilage correlates with worsening of symptoms¹² and predicts knee replacement.¹³ The emerging evidence suggests that there is a continuum from a normal knee to an osteoarthritic knee. This is further supported by a

recent study that showed that quantitative analysis of osteoarthritis by MRI is feasible using T and Z scores.¹⁴ A major advantage of using cartilage volume is that we can examine the state of knee cartilage as a continuous variable from the normal to the pre-diseased to the early diseased state.

In this study we examined a cohort of healthy middle aged men with no symptoms of knee osteoarthritis to examine change in tibial cartilage over two years, and to examine factors that may affect change.

METHODS

We followed up 28 eligible subjects (70%) of the original population of healthy white males recruited through advertising in newspapers, through sporting clubs, and through the staff association of the hospital.¹⁵ Exclusion criteria included previous significant knee injury requiring non-weight bearing treatment for more than 24 hours or surgery (including arthroscopy), evidence of radiographic osteoarthritis, osteoporosis, contraindication to MRI including pacemaker, metal sutures, presence of shrapnel, or iron filings in the eye. The study was approved by the ethics committee of the Royal Melbourne Hospital, Victoria, Australia.

Anthropometry

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)

Abbreviations: DHEAS, dehydroepiandrosterone sulphate; DPD, deoxypyridinoline corrected for creatinine; DXA, dual energy x ray absorptiometry; LH, luteinising hormone; Ntx, N-telopeptide cross links of type I collagen; PYD, pyridinoline corrected for creatinine; SHBG, sex hormone binding globulin; TBBMC, total body bone mineral content

Table 1 Characteristics of the 28 study subjects

Age (years) at baseline	51.9 (12.8)
Body mass index (kg/m ²) at baseline	24.9 (2.9)
Physical activity score at baseline	6.3 (1.8)
Baseline tibial cartilage volume (μl)	5513 (1201)
Annual loss of tibial cartilage volume (μl)	162 (93)
Tibial area (mm ²) at baseline	3342 (555)
<i>Hormone and metabolic bone and cartilage markers at baseline</i>	
Free testosterone (pmol/l)	370 (147)
Oestrogen (pmol/l)	62.1 (31.8)
Dehydroepianosterone sulphate (μmol/l)	8.6 (4.0)
Luteinising hormone (IU/l)	4.8 (2.1)
Pyridinoline/Cr (PYD) (nmol/mmol Cr)	53.9 (25.3)
Deoxypyridinoline/Cr (DPD) (nmol/mmol Cr)	8.6 (4.0)
N-telopeptide/Cr (NTx) (nmol bone collagen equivalents/mmol Cr)	47.3 (22.5)
Osteocalcin (ng/ml)	4.5 (1.9)

Values are mean (SD).
Cr, creatinine.

using a stadiometer. Body mass index (BMI) (weight/height², kg/m²) was calculated. Current total activity was a composite score of total amount of walking (0–4) + activity at home (0–4) + sporting activity (0–4).³

MRI

Each subject had MRI of their dominant knee, defined as the lower limb from which they stepped off when walking. The MRI was carried out at the beginning of the study and approximately two years later. Knee cartilage volume was determined as previously described.¹⁵ Knees were imaged in the sagittal plane on a 1.5 T whole body magnetic resonance unit (Signa Advantage HiSpeed GE Medical Systems, Milwaukee, Wisconsin, USA) using a commercial transmit–receive extremity coil. The following sequence and parameters were used: a T1 weighted fat suppressed three dimensional gradient recall acquisition in the steady state; flip angle 55°; repetition time 58 ms; echo time 12 ms; field of view 16 cm; 60 partitions; 512×196 matrix; one acquisition time 11 min 56 s. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31×0.08 mm (512×512 pixels). The image data were transferred to a workstation.

The volumes of the individual cartilage plates (medial and lateral tibial) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on each section. These data were resampled by bilinear and cubic interpolation (area of 312 and 312 μm and 1.5 mm thickness, continuous sections) for the final three dimensional rendering. The volume of the particular cartilage plate was determined by summing the pertinent voxels within the resultant binary volume. Two trained observers read each MRI independently and the average of their measurements was used. The coefficients of variation (CV) for medial and lateral tibial cartilage volumes were 2.6% and 3.3%, respectively. Tibial plateau area was determined by creating an isotropic volume from the three input images closest to the knee joint, which were reformatted in the axial plane. The area was directly measured from these images. The CV for the medial and lateral tibial plateau areas was 2.2% and 2.3%, respectively.⁸

Blood and urine analyses

Early morning blood samples and first void urine samples were collected at baseline from all subjects within eight weeks of their MRI. Serum and urine specimens were stored at –20°C. All laboratory results were analysed blind to the clinical data.

Total testosterone was measured by radioimmunoassay (DPC Coat-A-Count® total testosterone kit). Free testosterone in picomoles (pM) was calculated using the Sodergard equation.¹⁶ The intra-assay and interassay coefficients of variation were 9.8% and 15.6%, respectively.

Sex hormone binding globulin (SHBG) was measured by immunoradiometric assay (Orion® SHBG kit). The intra-assay and interassay coefficients of variation were 3.2% and 11.3%, respectively.

Dehydroepianosterone sulphate (DHEAS) was measured by a DPC Immulite® autoanalyser. The intra-assay and interassay coefficients of variation were 9.5% and 13%, respectively.

Oestradiol was measured by a DPC Immulite® autoanalyser. The intra-assay and interassay coefficients of variation were 4% and 5%, respectively.

Luteinising hormone (LH) was measured by Abbot AxSym® autoanalyser. The intra-assay and interassay coefficients of variation were 5.1% and 7.7%, respectively.

Total urine pyridinoline corrected for creatinine (PYD) was measured by high performance liquid chromatography (HPLC). The intra-assay and interassay coefficients of variation were 16% and 18.7%, respectively.

Total urine deoxypyridinoline corrected for creatinine (DPD) was measured by HPLC. The intra-assay and interassay coefficients of variation were 11.6% and 13%, respectively.

Urine N-telopeptide cross links of type I collagen (NTx) were measured by an Osteomark® enzyme linked immunosorbent assay (ELISA) (Ostex International, Seattle, Washington, USA). The intra-assay and interassay coefficients of variation were 7.2% and 6.5%, respectively.

Serum osteocalcin was measured by a two site immunoradiometric assay (Diagnostic Systems Laboratories Inc, Webster, Texas, USA). The intra-assay and interassay coefficients of variation were 3.1% and 5.3%, respectively.

Total body bone mineral content (TBBMC) was measured by dual energy x ray absorptiometry (DXA) using a Hologic QDR 1000W densitometer.

Statistical analysis

Annual change in tibial cartilage volume was calculated as [(baseline tibial cartilage volume μl) – (tibial cartilage volume at follow up μl)]/time between scans (in year), and also expressed as percentage change from baseline tibial cartilage volume (μl). We examined whether the annual percentage change in tibial cartilage volume was normally distributed using the Shapiro–Wilk test for normality, where the number of observations is less than 50 (p=0.48, indicating that our data were normally distributed). Multiple linear regression models were used to explore the possible associations between potential risk factors and change in cartilage volume, including age, BMI, bone size, and physical activity. Models included cartilage volume at baseline as a covariate to account for any regression to the mean artefact in the changes over time. We explored the association between initial cartilage volume and changes in tibial cartilage volume, adjusted for regression to the mean, using the method of Chuang-Stein and Tong.¹⁷ Annual change in tibial cartilage volume was regressed against various serum hormone levels and biochemical bone markers, adjusting for potential confounding variables. All analyses were carried out using the SPSS statistical package (version 10.0.5, SPSS, Cary, North Carolina, USA). Results are presented as mean (SD).

RESULTS

Twenty eight of the eligible men originally recruited completed the study (70%).¹⁵ Five men from the original

Table 2 Factors affecting longitudinal change in tibial cartilage volume in men over two years

Variable	Univariate analysis regression coefficient (95% CI)*	p Value	Multivariate analysis† regression coefficient (95% CI)*	p Value
Age	-1.8 (-13.9 to 10.4)	0.77	6.3 (-6.3 to 18.9)	0.31
BMI	-12.9 (-65.7 to 39.8)	0.62	-16.3 (-70.5 to 37.9)	0.54
Physical activity	-6.3 (-84.2 to 71.6)	0.87	-25.0 (-116.7 to 66.6)	0.57
Tibial plateau area	0.01 (-0.33 to 0.35)	0.96	0.03 (-0.35 to 0.41)	0.85

*Change in cartilage per unit increase in corresponding variable

†Multivariate analysis with age, BMI, physical activity, baseline tibial cartilage volume, and tibial plateau area in regression equation.

BMI, body mass index; CI, confidence interval.

study were excluded as they had osteoarthritis or osteoporosis, one died, three were overseas, three refused to participate, and five were not contactable. The demographic features and baseline hormonal status of the study participants who completed the study are presented in table 1. When the non-participants are compared with participants, no significant differences were observed for the following characteristics: age (mean age 52.3 years, $p=0.93$ for difference), BMI (mean 26.7 kg/m², $p=0.21$ for difference), initial tibial cartilage volume (mean 5312 μl , $p=0.95$ for difference), tibial plateau area (mean 3697 μm^2 , $p=0.67$ for difference), or presence of symptoms. When the non-participants are compared to participants, no significant differences were observed for age, BMI, initial cartilage volume, tibial plateau area, or presence of symptoms.

The mean (SD) reduction in tibial cartilage volume per year was 162 (93) μl (table 1). When expressed as annual percentage change in cartilage volume, the mean reduction in cartilage volume was 2.8% of total tibial cartilage per year (95% confidence interval (CI), 0.2% to 5.5%). Possible associations between potential risk factors and change in cartilage in the men were explored. There was no significant association between tibial cartilage loss and age, BMI, or level of physical activity (table 2).

Testosterone was associated with loss of tibial cartilage in univariate analysis and also following adjustment for age, BMI, baseline tibial cartilage volume tibial plateau area and total bone mineral content (table 3). Total bone mineral content was included in the regression to adjust for a potential effect of testosterone on bone. No such association

was seen for DHEAS, LH, or oestrogen. Overall, testosterone accounted for 14.5% (partial r^2) of the variation in change in tibial cartilage volume. This remained unchanged after adjustment for serum oestrogen levels.

When biochemical bone turnover markers were examined, there was a trend for urine N-telopeptide cross links of type I collagen (NTx) to be associated with tibial cartilage loss in the univariate analysis ($p=0.07$) (table 3). This effect persisted after adjusting for age, BMI, baseline tibial cartilage volume, tibial plateau area, and total bone mineral content ($p=0.057$). No significant association was observed between tibial cartilage loss and urinary pyridinoline, deoxypyridinoline, or osteocalcin in either the univariate or the multivariate analyses.

DISCUSSION

We found that in a group of 28 healthy men, tibial cartilage was lost over two years at an average rate of 2.8% per year. While these data suggest a reduction in cartilage volume over time in healthy men, based on the 95% confidence intervals, the average annual reduction may be as low as 0.2% a year or even as high as 5.5%. We found that tibial cartilage loss was associated with serum free testosterone level. The testosterone levels in these men were within the normal range, with no subject having low levels. There was a trend towards a positive association between tibial cartilage loss and urinary NTx.

The only published longitudinal data on average change in cartilage in healthy men came from a small series ($n=13$).¹⁸ This series included both men ($n=5$) and women ($n=8$),

Table 3 Relation between longitudinal change in tibial cartilage volume in men over two years and sex hormones and metabolic bone markers

Variable	Univariate analysis regression coefficient (95% CI)*	p Value	Multivariate analysis† regression coefficient (95% CI)*	p Value
<i>Hormones</i>				
Free testosterone	1.12 (0.21 to 2.03)	0.02	1.16 (0.09 to 2.23)	0.036
Sex hormone binding globulin		0.91		0.98
Oestrogen	-0.6 (-11.3 to 10.1)	0.81	-0.22 (-16.2 to 15.7)	0.24
Dehydroepiandrosterone sulphate	0.8 (-5.9 to 7.5)	0.51	3.6 (-2.64 to 9.78)	0.57
Luteinising hormone	22.3 (-46.6 to 91.4)	0.21	29.7 (-79.2 to 138.7)	0.28
	51.9 (-31.8 to 135.6)		48.3 (-43.3 to 139.8)	
<i>Biochemical bone turnover markers</i>				
Pyridinoline (PYD)	3.5 (-2.0 to 9.0)	0.20	1.9 (-3.4 to 7.3)	0.46
Deoxypyridinoline (DPD)	14.0 (-22.5 to 50.5)	0.43	7.5 (-29.6 to 44.7)	0.68
N-telopeptide (NTx)	19.4 (-2.1 to 40.9)	0.07	15.1 (1.5 to 28.7)	0.057
Osteocalcin	-47.1 (-128.0 to 33.9)	0.24	-55.4 (-149.1 to 38.4)	0.23

*Change in cartilage per unit increase in corresponding variable.

†Multivariate analysis with age, body mass index, baseline tibial cartilage volume, tibial plateau area, and total bone mineral content in regression equation.

CI, confidence interval.

and involved a younger population (mean age, 49 (18) years) than the one we describe here.¹⁸ This showed average annual cartilage gain. However, the 95% confidence interval for average annual change in cartilage (gain of 6.7% per year, loss of 2.3% per year) overlapped those of this study in healthy adults. We have recently published a longitudinal study of healthy women. In that study we showed that the mean tibial cartilage volume loss in healthy postmenopausal women was between 1.5% and 3.2% a year, similar to the findings in this study.⁹ In contrast to these studies in healthy subjects, subjects with osteoarthritis lose tibial cartilage at an average annual rate of 5.3% (95% CI, 4.4% to 6.2%), approximately double the rate observed in these healthy men.⁹

We showed that loss of cartilage in this healthy population of middle aged men lay between 0.2% and 5.5% a year, with the average change being 2.8% of total tibial cartilage per year. At the level of the individual, the minimum detectable change that can be distinguished from measurement error (at a 5% level of significance) is 2.8 multiplied by the coefficient of variation for an individual volume measurement—that is, approximately 8% for total tibial cartilage volume in this study.¹⁹ Thus no individual had a change in cartilage volume that was outside the limits of measurement error, at the individual level.¹⁹ Despite this, it may be that some subjects truly gained cartilage over the course of the study or that swelling occurred in existing cartilage. Only improvements in measurement technique, including developing the ability to identify normal cartilage, will answer this question. Indeed, it is also accepted that this estimate of the minimal detectable difference may underestimate the confidence intervals of the measurement.

Few data are available on the effect of testosterone on joint cartilage or osteoarthritis, despite knowledge of its effects on other musculoskeletal tissues.²⁰ Human chondrocytes possess testosterone receptors on their surface.²¹ We previously showed that the serum free testosterone level was associated with tibial cartilage volume in a cross sectional study in healthy men. However, having followed these men prospectively, we have found that serum free testosterone is associated with an increased rate of cartilage loss. This appears to be independent, and indeed in contrast to the effect of testosterone on bone.²² It may be that, although a higher serum testosterone is associated with achieving a greater amount of knee cartilage, this is not of long term benefit, as the rate of cartilage loss associated with higher free testosterone is also greater. It appears that those who have a greater amount of cartilage related to a higher serum testosterone also lose it at a greater rate when followed longitudinally. This may simulate the effect of exercise on bone mineral density. For instance, in soccer players, bone mineral density increases with sport participation, but then falls more rapidly to reach a level not higher than those who did not participate in sport.²³ Alternatively, it maybe that higher testosterone levels also have an indirect effect on cartilage through increased musculoskeletal activity, resulting in greater biomechanical forces in joints and increasing the level of degradation of articular cartilage.

In this study we found a trend for NTx—a urinary cross linked N-telopeptides of type I collagen, a marker of bone resorption—to be associated with tibial cartilage loss, independent of total body bone mineral content. This is consistent with a previous study that found that bone resorption as measured by NTx was increased in patients with progressive knee osteoarthritis measured radiologically but not in those with non-progressive knee osteoarthritis.²⁴ The lack of any such relation with deoxypyridinoline and pyridinoline may be explained by the fact that the NTx molecule is more specific to bone, owing to the unique amino

acid sequence and orientation of the cross linked α -2 N-telopeptide,²⁵ and clinical studies suggest that amino- or carboxy-terminal telopeptides provide the most responsive and specific indicator of the bone resorption process of all currently known markers.^{25–27} The coefficients of variation for NTx were also less than for other bone resorption markers. The increase in bone resorption, associated with knee cartilage loss, suggests that the alterations in bone turnover may be important in the pathogenesis of knee osteoarthritis and warrants further investigation as a possible therapeutic target in the prevention of knee osteoarthritis.

A limitation of our study is that we only included men. Our findings cannot be generalised to women as the associations we found may be sex specific. However, we have previously reported the rate of change in tibial cartilage in healthy women.⁹ We examined men and women separately as significant gender effects have been described in knee cartilage.⁷ This study of only 28 subjects, with limited range of age, BMI, and physical activity scores, had little power to show an effect of these variables on cartilage loss. In this study we excluded men who had osteoarthritis or osteoporosis. We have previously reported that subjects with knee osteoarthritis lose cartilage at 5.3% per annum, which was approximately the rate we found in normal women.⁸ There is also evidence that bone changes may be important in the pathogenesis of osteoarthritis²⁸ and that there is an inverse association between osteoarthritis and osteoporosis.²⁹ These may confound any relations between sex hormones and metabolic bone markers, which we examined in this study. Our study was potentially limited by a relatively small sample size. Although we were able to show a significant effect of serum testosterone on tibial cartilage loss, we cannot exclude the presence of weaker relations between loss of tibial cartilage and risk factors such as BMI and physical activity. A strength of this study is that we used a novel method for assessing knee cartilage volume in healthy asymptomatic subjects.

It is possible that the men who were lost to follow up lost cartilage at a different rate to those who completed the study. However, these men did not differ in any measurable way from those that completed the study, and did not withdraw from the study for reasons related to their knees, such as the presence or absence of knee symptoms. Our method of measuring cartilage volume has been validated against cadaveric and postoperative specimens, using water displacement, and has excellent reproducibility (2–3%).⁷ In this study we only measured tibial cartilage, having previously shown that tibial and femoral cartilage volumes³⁰ and change in tibial and femoral cartilage³¹ are correlated. Our study has small numbers and so has limited ability to detect effects on change in cartilage volume. This may explain a lack of effect of other bone markers and some of the other variables.

Conclusions

In healthy men tibial cartilage volume is lost at an average rate of 2.8% per year. The serum testosterone level at baseline and urinary NTx, a marker of bone turnover, were inversely related to cartilage loss. Further studies will be required to determine whether hormonal manipulation or treatment with antiresorptive drugs will reduce the risk of knee osteoarthritis in men in later life.

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