ARTHRITIS & RHEUMATISM
Vol. 60, No. 5, May 2009, pp 1381–1389
DOI 10.1002/art.24486

Serum Levels of Vitamin D, Sunlight Exposure, and Knee Cartilage Loss in Older Adults

The Tasmanian Older Adult Cohort Study

Changhai Ding,[1] Flavia Cicuttini,[2] Venkat Parameswaran,[3] John Burgess,[4] Steve Quinn,[5] and Graeme Jones[5]

Objective. **To determine the associations between serum levels of vitamin D, sunlight exposure, and knee cartilage loss cross-sectionally and longitudinally in older adults.**

Methods. **A total of 880 randomly selected subjects (mean age 61 years [range 51–79 years], 50% women) were studied at baseline, and 353 of these subjects were studied 2.9 years later. Serum levels of 25-hydroxyvitamin D (25[OH]D) were assessed by radioimmunoassay, and sunlight exposure was assessed by questionnaire. T1-weighted fat-suppressed magnetic resonance imaging (MRI) of the right knee was performed to determine knee cartilage volume and defects. Knee radiographic osteoarthritis (OA) and knee pain were also assessed.**

Results. **The mean 25(OH)D serum level was 52.8 nmoles/liter at baseline (range 13–119 nmoles/liter). Winter sunlight exposure and serum 25(OH)D level were both positively associated with medial and lateral tibial cartilage volume, and a serum 25(OH)D level <50 nmoles/liter was associated with increased medial tibiofemoral joint space narrowing (all $P < 0.05$). Longitudinally, baseline serum 25(OH)D level predicted change in both medial and lateral tibial cartilage volume ($\beta = +0.04\%$ per annum per nmole/liter for both; $P < 0.05$), and change in serum 25(OH)D level was positively associated with change in medial tibial cartilage volume. These associations were consistent in subjects with radiographic OA and knee pain and/or in women, but not in men or in subjects without radiographic OA or knee pain.**

Conclusion. **Sunlight exposure and serum 25(OH)D levels are both associated with decreased knee cartilage loss (assessed by radiograph or MRI). This is best observed using the whole range of 25(OH)D levels rather than predefined cut points and implies that achieving vitamin D sufficiency may prevent and/or retard cartilage loss in knee OA.**

Both osteoarthritis (OA) and vitamin D insufficiency are common health conditions in older people. Approximately 25% of people age >55 years have had knee pain on most days in a month in the past year, of which about half have radiographic knee OA and thus are considered to have symptomatic OA (1). Furthermore, it has been estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency (2,3). Vitamin D insufficiency has been linked with osteoporosis and fractures both in older women and in older men (2); however, the role of vitamin D insufficiency in the pathogenesis of OA is controversial.

Preliminary evidence suggests that vitamin D has direct effects on chondrocytes in OA cartilage, and

Supported by the National Health and Medical Research Council of Australia, the Arthritis Foundation of Australia, the Tasmanian Community Fund, and the University of Tasmania (Institutional Research Scheme grant). Dr. Ding is recipient of a National Health and Medical Research Council of Australia Clinical Career Development Award. Dr. Jones is recipient of a National Health and Medical Research Council of Australia Practitioner Fellowship.

[1]Changhai Ding, MD: University of Tasmania, Hobart, Tasmania, and Monash University Medical School, Melbourne, Victoria, Australia; [2]Flavia Cicuttini, PhD: Monash University Medical School, Melbourne, Victoria, Australia; [3]Venkat Parameswaran, MSc, PhD, ARCPA: Royal Hobart Hospital, Hobart, Tasmania, Australia; [4]John Burgess, MD, PhD, FRACP: University of Tasmania, and Royal Hobart Hospital, Hobart, Tasmania, Australia; [5]Steve Quinn, PhD, Graeme Jones, MD, FRACP: University of Tasmania, Hobart, Tasmania, Australia.

Address correspondence and reprint requests to Changhai Ding, MD, Menzies Research Institute, Private Bag 23, Hobart, Tasmania 7000, Australia. E-mail: changhai.ding@utas.edu.au.

Submitted for publication September 29, 2008; accepted in revised form February 5, 2009.

vitamin D receptors have been demonstrated in human articular chondrocytes of OA cartilage, especially in the superficial zone (4,5). Vitamin D may also exert an effect on OA through bone, and vitamin D insufficiency could impair the ability of bone to respond optimally to insults, thus predisposing to disease progression (4). Although 2 epidemiologic studies have shown that vitamin D deficiency was associated with an increased risk of progression of knee OA (both joint space narrowing [JSN] and osteophytes) (6) and incidence of hip OA (JSN but not osteophytes) (7), other clinical studies have shown no association between serum vitamin D levels and joint space loss (8,9).

Most of these studies have used radiographic assessment of OA, which is 2-dimensional (2-D) in nature, lacks sensitivity to change, and is susceptible to measurement error through factors such as joint positioning. Investigators in 1 study (8) assessed cartilage defects with magnetic resonance imaging (MRI) and reported that serum vitamin D was not associated with knee focal cartilage loss, but they did not assess the whole knee or cartilage volume. Using sensitive methods (such as quantitative assessment of cartilage volume by MRI [10,11]) to reveal the associations between vitamin D and knee OA structural changes has the potential to clarify this area. Therefore, the aim of this prospective study was to determine whether serum levels of vitamin D and sunlight exposure were associated with knee cartilage volume both cross-sectionally and longitudinally in older adults.

PATIENTS AND METHODS

Subjects. The Tasmanian Older Adult Cohort (TASOAC) study is an ongoing prospective study in southern Tasmania. Baseline measurements were conducted from April 2002 to September 2004, and the first followup was from September 2004 to February 2007. Subjects (98% Caucasian) ages 50–80 years were selected randomly using computer-generated random numbers from the roll of electors in southern Tasmania (population 229,000), a comprehensive population listing, with an equal number of men and women selected as subjects. We excluded institutionalized persons, subjects with contraindication to MRI (including metal sutures, presence of shrapnel, iron filings in the eye, and claustrophobia), and subjects with rheumatoid arthritis. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee, and written informed consent was obtained from all participants.

Anthropometrics and questionnaire. Height was measured to the nearest 0.1 cm (with shoes, socks, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Delta Model 707; Seca, Hamburg, Germany) that were calibrated using a known weight at the beginning of each clinic. Body mass index (BMI; weight [kg]/height [m^2]) was calculated. Self-report of smoking status and disease status such as asthma, cardiovascular disease, and diabetes were recorded by questionnaire.

Sunlight exposure and steps per day. Sunlight exposure was assessed by questionnaire relating to the amount of daily exposure during weekdays, weekends, and on holidays in winter or summer. Categories were as follows: 1 = <1 hour; 2 = 1–<2 hours; 3 = 2–<3 hours; 4 = 3–<4 hours; and 5 = ≥4 hours. This measure of exposure against actual exposure with polysulfone badges has been previously validated in teenagers with an intraclass correlation coefficient (ICC) of 0.62 (12). Steps per day were assessed using the HJ-002 pedometer (Omron, Tokyo, Japan) for 1 week at baseline.

Serum vitamin D measurement. Serum samples were treated initially with acetonitrile to rapidly extract 25-hydroxyvitamin D (25[OH]D) and other hydroxylated metabolites. We then assayed 25(OH)D using a Liquid Phase radioimmunoassay (IDS, Boldon, Tyne & Wear, UK). The intra- and interassay coefficients of variation (CVs) we obtained were 1.8% and 3.3%, respectively. Vitamin D insufficiency was defined as a 25(OH)D concentration <50 nmoles/liter (12). Change in vitamin D concentration was calculated as follows: change per annum = (followup vitamin D concentration – baseline vitamin D concentration)/time between the 2 measurements in years. The season of blood sampling was recorded.

Knee radiographs and knee pain assessments. A standing anteroposterior semiflexed view of the right and left knee with 15° of fixed knee flexion was performed in all subjects at baseline and scored individually for osteophytes and JSN on a scale of 0–3 (0 = normal and 3 = severe) according to the Osteoarthritis Research Society International atlas (13) as previously described (14). The presence of radiographic OA was defined as any score of ≥1.

Knee pain (on a flat surface, going up/down stairs, at night, sitting/lying, and standing upright) was assessed by self-administered questionnaire using the Western Ontario and McMaster Universities Osteoarthritis Index with a 10-point scale from 0 (no pain, stiffness, or no function problems) to 9 (most severe pain, stiffness, or severe function problems) (15). Each component of knee pain was summed to create a total pain (0–45) score. Prevalent knee pain was defined as a total score of ≥1.

Knee cartilage volume and defects assessments. MRI scans of the right knees were performed at baseline and followup. Knees were imaged in the sagittal plane on a 1.5T whole-body MR unit (Picker, Cleveland, OH), and a fat-suppressed T1-weighted spoiled gradient-echo sequence was used. Knee cartilage volume was determined by means of image processing on an independent workstation as previously described (11,16). Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 × 0.31 mm (512 × 512 pixels). The volumes of individual cartilage plates (medial tibial and lateral tibial) were isolated from the total volume of cartilage by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis. These data were then resampled by means of bilinear and cubic interpolation (area of 312 μm × 312 μm and 1.5-mm thickness, continuous sections) for the final 3-D ren-

Table 1. Characteristics of the participants*

	Vitamin D ≥50 nmoles/liter	Vitamin D <50 nmoles/liter	*P*
Baseline†			
Age, years	61.7 ± 7.0	62.6 ± 7.7	0.09
Women, %	45	56	0.001
BMI, kg/m^2	27.2 ± 3.9	28.3 ± 5.4	<0.001
Smokers, %	53	48	0.14
Knee pain, %	48	52	0.25
Winter sunlight exposure, 1–5	2.9 ± 1.4	2.3 ± 1.2	<0.001
Summer sunlight exposure, 1–5	3.1 ± 1.4	2.6 ± 1.3	<0.001
Right knee JSN, %	58	57	0.83
Right knee osteophytes, %	9	10	0.79
Left knee JSN, %	61	61	0.90
Left knee osteophytes, %	8	11	0.27
Prevalent total cartilage defects, %	52	55	0.47
Medial tibial cartilage volume, ml	2.4 ± 0.6	2.2 ± 0.6	<0.001
Lateral tibial cartilage volume, ml	2.9 ± 0.7	2.6 ± 0.7	<0.001
Medial tibial bone area, cm^2	21.2 ± 3.1	20.6 ± 3.0	0.007
Lateral tibial bone area, cm^2	12.4 ± 2.2	11.9 ± 2.2	0.002
25(OH)D level, nmoles/liter	66.4 ± 13.4	35.9 ± 8.4	<0.001
Changes per annum over 2.9 years‡			
25(OH)D level, nmoles/liter	−1.1 ± 7.9	3.8 ± 6.4	<0.001
Medial tibial cartilage volume, %	−2.1 ± 5.6	−2.9 ± 5.2	0.21
Lateral tibial cartilage volume, %	−1.7 ± 4.1	−2.1 ± 4.6	0.39

* Except where indicated otherwise, values are the mean ± SD. BMI = body mass index; JSN = joint space narrowing; 25(OH)D = 25-hydroxyvitamin D.
† At baseline, there were 488 subjects in the group with vitamin D levels ≥50 nmoles/liter and 392 subjects in the group with vitamin D levels <50 nmoles/liter.
‡ After 2.9 years, there were 194 subjects in the group with vitamin D levels ≥50 nmoles/liter and 159 subjects in the group with vitamin D levels <50 nmoles/liter.

dering. The volume of the particular cartilage plate was then determined by summing all the pertinent voxels within the resultant binary volume. The CVs we obtained for cartilage volume measures were 2.1–2.6% (16). Rates of change in cartilage volume were calculated as follows:

$$\text{Percentage change per annum} = 100 \times \frac{(\text{Followup volume} - \text{baseline volume})/\text{baseline volume}}{\text{Time between the 2 scans in years}}$$

Cartilage defects on a 0–4 scale were graded at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites as follows (17–19): grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface and bottom; grade 2 = irregularities on the surface or bottom and loss of thickness of <50%; grade 3 = deep ulceration with loss of thickness of >50%; grade 4 = full-thickness chondral wear with exposure of subchondral bone. A cartilage defect also had to be present in at least 2 consecutive slices. A prevalent cartilage defect was defined as a cartilage defect score of ≥2 at any site within that compartment. Intraobserver reliability (expressed as ICC) was 0.89–0.94, and interobserver reliability was 0.85–0.93 (18). An increase in cartilage defects was defined as a change in cartilage defects ≥1.

Tibial bone area at the medial and lateral compartments was determined as previously described (14). To transform the images from the sagittal to the axial plane, we used the Analyze Software package developed by the Mayo Clinic (Rochester, MN). Medial and lateral tibial plateau bone area was determined by creating an isotropic volume from the 3 input images closest to the knee joint. The bone area of the medial and lateral tibial plateau was then directly measured from the reformatted axial images. The CVs we obtained for these measures were 2.2–2.6%.

Statistical analysis. We used *t*-tests to compare means and Mann-Whitney U tests to compare proportions. A scatterplot and Spearman correlations were used to depict the association between unadjusted vitamin D levels and knee cartilage volume in the total sample as well as stratification by sex. Standard diagnostic checks of model fit and residuals were routinely made, and data points with large residuals and/or high influence were investigated for data errors. Vitamin D concentration and change in vitamin D concentration were not normally distributed ($P = 0.04$ and $P = 0.001$, respectively, by Kolmogorov-Smirnov test), but no transformations (log, square, and cubic) provided better results; furthermore, the residuals were investigated for both heteroscedasticity and normality, and there was no evidence of violation of the model assumptions, so we kept the nontransformed data as well as vitamin D insufficiency (yes versus no) and tertiles of change in vitamin D levels as independent variables in the regression analyses.

Univariable and multivariable linear regression analyses were used to examine the associations between knee cartilage volume (or change in knee cartilage volume) and vitamin D concentration (or sunlight exposure, change in

Table 2. Associations between vitamin D levels, sun exposure, and knee cartilage volume: cross-sectional data*

	Univariable β (95% CI)	Multivariable β (95% CI)†
25(OH)D level, <50 nmoles/liter vs. ≥50 nmoles/liter		
Medial tibial	−200 (−280, −118)‡	−114 (−186, −42)‡
Lateral tibial	−256 (−350, −162)‡	−121 (−200, −43)‡
25(OH)D level, per nmole/liter		
Medial tibial	+6.2 (+4.1, +8.3)‡	+2.4 (+0.7, +4.2)‡
Lateral tibial	+7.5 (+5.1, +10.0)‡	+2.8 (+0.8, +4.9)‡
Winter sun exposure, per grade		
Medial tibial	+112 (+82, +141)‡	+38 (+10, +67)‡
Lateral tibial	+156 (+122, +191)‡	+57 (+26, +88)‡
Summer sun exposure, per grade		
Medial tibial	+95 (+66, +124)‡	+22 (−5, +49)
Lateral tibial	+130 (+97, +163)‡	+29 (+1, +57)‡

* The dependent variable is cartilage volume in mm^3. 95% CI = 95% confidence interval (see Table 1 for other definitions).
† Adjusted for age, sex, BMI, smoking, tibial bone area, JSN, osteophytes, season of blood sampling, steps per day, knee pain, asthma, cardiovascular diseases, and diabetes.
‡ $P < 0.05$.

vitamin D concentration) before and after adjustment for age, sex, BMI, smoking, steps per day, knee pain, cartilage defects, season of blood sampling, tibial bone area, and radiographic OA and/or other diseases (cardiovascular disease, asthma, and diabetes). The associations among knee radiographic changes (JSN or osteophytes) and vitamin D insufficiency and sunlight exposure, both before and after adjustment for the above factors, were analyzed by logistic regression using generalized estimating equations (GEEs). GEEs account for the fact that data from both knees are correlated. An exchangeable correlation matrix was used, since observations are clustered (and not time dependent), and all models used robust variance estimates to ensure valid standard errors. A P value less than 0.05 (2-tailed) or a 95% confidence interval not including the null point was regarded as statistically significant. All statistical analyses were performed using SPSS version 12.0 for Windows (SPSS, Chicago, IL) or Stata statistical software: release 10.0 (College Station [TX]: Stata Corporation; 2006).

RESULTS

A total of 1,002 subjects (50% women) ages 51–79 years (mean 61 years) participated in the TASOAC study. Of those 1,002 subjects, 122 had no knee MRI scans at baseline (43 had contraindications to MRI [33 had claustrophobia, 8 had metal in the body, and 2 had a pacemaker], 5 had joint replacements, and the rest refused or were unable to undergo the procedure). Over 2.9 years, 254 subjects were lost to followup (122 had no MRI at baseline [as mentioned above], 28 were deceased, 20 moved to other states or overseas, 15 had joint replacements, 28 were physically unable, and others refused or provided no reasons). In 748 subjects (75% of those originally studied) who completed the study, the first 353 had the second MRI scans but the others did not, because the local MRI machine was updated and became unavailable for research purposes. The rate of vitamin D supplementation in this cohort was 4%. There were no significant differences in demographic factors, 25(OH)D levels, and knee cartilage volume between the subjects who did and those who did not have the second MRI scans (mean ± SD age 62.6 ± 7.5 years versus 62.2 ± 7.5 years; $P = 0.41$) (50% women in both groups; $P = 0.87$) (mean ± SD BMI 27.6 ± 4.4 kg/m^2 versus 27.8 ± 4.8 kg/m^2; $P = 0.57$) (mean ± SD 25[OH]D concentration 52.8 ± 18.8 nmoles/liter versus 52.3 ± 18.8 nmoles/liter; $P = 0.64$) (mean ± SD total cartilage volume 8.3 ± 1.9 ml versus 8.2 ± 2.0 ml; $P = 0.59$).

Fifty-eight percent of the subjects had radiographic changes (JSN or osteophyte score of ≥1) in the right knee, and 50% had knee pain. The mean 25(OH)D level in this sample was 52.8 nmoles/liter at baseline (range 13–119 nmoles/liter), and the prevalence of vitamin D insufficiency was 45%. The mean change in 25(OH)D level per annum, which is associated with sunlight exposure and change in season (data not shown), was +0.91 nmoles/liter (range –26 nmoles/liter to +27 nmoles/liter). Characteristics of the subjects are presented in Table 1. Subjects with vitamin D levels ≥50 nmoles/liter and <50 nmoles/liter were similar in terms of smoking status, JSN and osteophytes at both the right and left knees, and prevalent cartilage defects. However, subjects with low vitamin D levels were older, more likely to be women, and had higher BMI, less sun exposure, and lower knee cartilage volume. Moreover,

BMI (odds ratio [OR] 1.05 per unit, $P < 0.001$) and female sex (OR 1.61, $P < 0.001$) were independently associated with vitamin D insufficiency. Tibial bone area was lower in subjects with vitamin D insufficiency, but this was due to the higher proportion of women.

At baseline, those with vitamin D insufficiency had lower knee cartilage volume at both medial and lateral tibial sites in the univariable and multivariable analyses in the total sample (Table 2). This was true in women ($P < 0.01$), men ($P < 0.05$), subjects with radiographic OA ($P < 0.01$), subjects without radiographic OA ($P < 0.01$), subjects with knee pain ($P < 0.01$), and subjects without knee pain ($P < 0.01$). There was no significant interaction between sex and vitamin D insufficiency, radiographic OA and vitamin D insufficiency, and knee pain and vitamin D insufficiency for medial and lateral tibial cartilage volume (all $P > 0.05$). When analyzed as a continuous variable, 25(OH)D levels were positively associated with knee cartilage volume at both tibial sites in all analyses (Figure 1 and Table 2). Sunlight exposure, which correlated significantly with serum 25(OH)D levels in this sample (both $\rho = +0.25$ for winter and summer sunlight exposure, $P < 0.001$), was also significantly associated with tibial cartilage volume in multivariable analyses (Table 2). Surprisingly, the associations between sunlight exposure and cartilage volume decreased in magnitude but remained significant after further adjustment for 25(OH)D levels. In adjusted analysis, 25(OH)D insufficiency was weakly and positively associated with medial tibial bone area (yes versus no: $\beta = 29$ mm^2, $P = 0.12$) and lateral tibial bone area (yes versus no: $\beta = 21$ mm^2, $P = 0.07$) in women, but not in men.

Vitamin D levels at baseline predicted change in both medial and lateral tibial cartilage volume, and vitamin D insufficiency also predicted change in medial tibial cartilage volume after adjustment for listed potential confounders (Table 3). Changes in 25(OH)D levels as a continuous variable and as tertiles were also significantly associated with change in medial tibial cartilage volume but not with change in lateral tibial cartilage volume in multivariable analysis (Table 3). The significant associations among baseline 25(OH)D levels, change in 25(OH)D levels, and change in tibial cartilage volume were consistent in subjects with radiographic OA and knee pain and/or in women, but not in men or in subjects without radiographic OA or knee pain (Table 4). The effects of interactions between sex and 25(OH)D levels ($P = 0.19$), knee pain and 25(OH)D levels ($P = 0.43$), and right medial radiographic OA and 25(OH)D levels ($P = 0.16$) on loss of medial tibial cartilage volume were all nonsignificant, as were the effects of interactions between sex and 25(OH)D levels ($P = 0.90$), knee

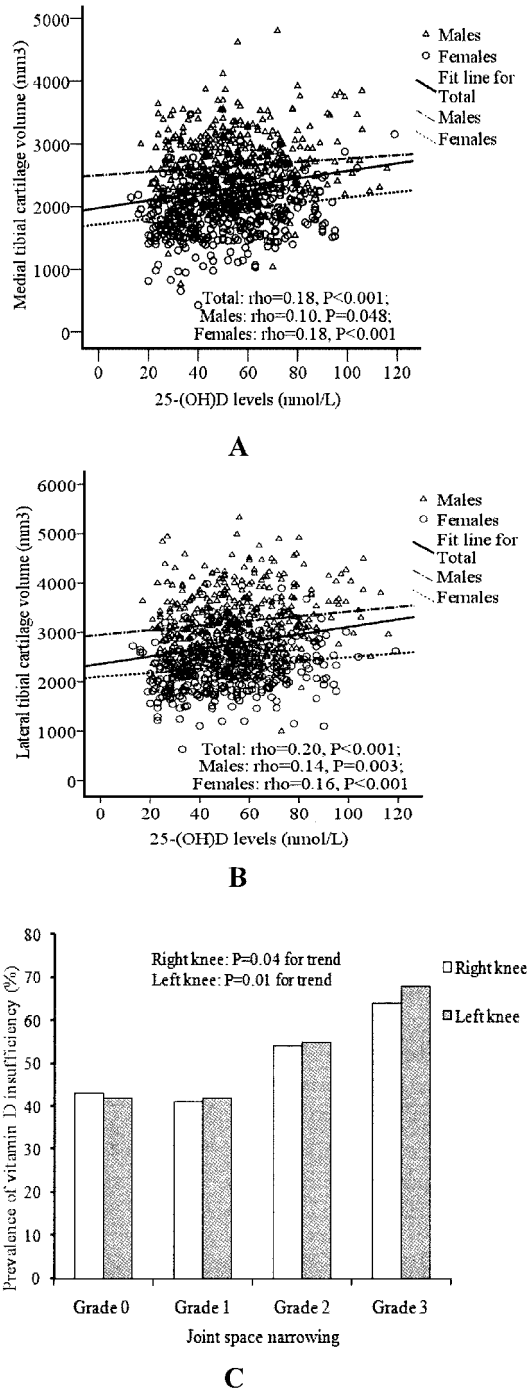

Figure 1. Association of serum levels of 25-hydroxyvitamin D (25[OH]D) with medial tibial cartilage volume (**A**), lateral tibial cartilage volume (**B**), and joint space narrowing (JSN) (**C**). JSN was scored on a scale of 0–3 (0 = normal and 3 = severe).

Table 3. Associations between vitamin D level at baseline, change in vitamin D level, and change in knee cartilage volume: longitudinal data*

	Multivariable β (95% CI)†	Multivariable β (95% CI)‡
Baseline 25(OH)D level, per nmole/liter		
Medial tibial	+0.03 (+0.00, +0.06)§	+0.04 (+0.01, +0.07)§
Lateral tibial	+0.02 (−0.00, +0.05)	+0.04 (+0.01, +0.07)§
Baseline 25(OH)D level, <50 nmoles/liter vs. ≥50 nmoles/liter		
Medial tibial	−1.31 (−2.45, −0.17)§	−1.46 (−2.61, −0.31)§
Lateral tibial	−0.55 (−1.54, +0.43)	−0.77 (−1.80, +0.25)
Change in 25(OH)D level, per nmole/liter		
Medial tibial	+0.06 (−0.04, +0.16)	+0.10 (+0.01, +0.20)§
Lateral tibial	+0.02 (−0.07, +0.10)	+0.04 (−0.04, +0.12)
Change in 25(OH)D level, per tertile		
Medial tibial	+0.61 (−0.12, +1.34)	+0.90 (+0.17, +1.63)§
Lateral tibial	+0.07 (−0.56, +0.70)	+0.20 (−0.46, +0.85)

* The dependent variable is change in cartilage volume, % per annum. 95% CI = 95% confidence interval (see Table 1 for other definitions).
† Adjusted for baseline cartilage volume and/or baseline vitamin D level (for change in vitamin D level).
‡ Further adjusted for age, sex, BMI, smoking, tibial bone area, cartilage defects, knee pain, season of blood sampling, JSN, osteophytes, sun exposure, steps per day, asthma, cardiovascular diseases, and diabetes.
§ $P < 0.05$.

pain and 25(OH)D levels ($P = 0.72$), and right lateral radiographic OA and 25(OH)D levels ($P = 0.69$) on loss of lateral tibial cartilage volume. The effects of interactions between 25(OH)D levels and radiographic OA status (no radiographic OA [33%], unilateral radiographic OA [13%], and bilateral radiographic OA [54%]) on loss of medial ($P = 0.10$) and lateral ($P = 0.64$) tibial cartilage volume were also nonsignificant.

Cross-sectionally, vitamin D levels were not associated with JSN of the right knee ($\beta = -0.51$ unit per grade, $P = 0.60$) or left knee ($\beta = -1.20$ unit per grade, $P = 0.12$), but vitamin D insufficiency was significantly

Table 4. Associations between vitamin D level at baseline, change in vitamin D level, and change in knee cartilage volume: subgroup analyses*

	Medial tibial β (95% CI)†	Lateral tibial β (95% CI)†
Women		
Baseline 25(OH)D level, per nmole/liter	+0.07 (+0.02, +0.12)‡	+0.04 (−0.00, +0.08)
Change in 25(OH)D level, per nmole/liter	+0.15 (+0.00, +0.29)‡	+0.06 (−0.08, +0.19)
Men		
Baseline 25(OH)D level, per nmole/liter	+0.01 (−0.04, +0.06)	+0.04 (0.00, +0.08)
Change in 25(OH)D level, per nmole/liter	+0.04 (−0.11, +0.20)	+0.05 (−0.06, +0.16)
Subjects with radiographic OA		
Baseline 25(OH)D level, per nmole/liter	+0.08 (+0.03, +0.12)‡	+0.06 (+0.02, +0.10)‡
Change in 25(OH)D level, per nmole/liter	+0.14 (+0.01, +0.27)‡	+0.05 (−0.07, +0.17)
Subjects without radiographic OA		
Baseline 25(OH)D level, per nmole/liter	+0.03 (−0.03, +0.08)	+0.02 (−0.03, +0.07)
Change in 25(OH)D level, per nmole/liter	+0.10 (−0.07, +0.27)	+0.01 (−0.10, +0.12)
Subjects with knee pain		
Baseline 25(OH)D level, per nmole/liter	+0.06 (+0.01, +0.12)‡	+0.05 (+0.01, +0.10)‡
Change in 25(OH)D level, per nmole/liter	+0.26 (+0.08, +0.43)‡	+0.07 (−0.07, +0.22)
Subjects without knee pain		
Baseline 25(OH)D level, per nmole/liter	+0.03 (−0.01, +0.07)	+0.03 (−0.01, +0.06)
Change in 25(OH)D level, per nmole/liter	−0.03 (−0.15, +0.09)	−0.01 (−0.11, +0.09)

* The dependent variable is change in cartilage volume, % per annum. 95% CI = 95% confidence interval (see Table 1 for other definitions).
† Adjusted for baseline cartilage volume and/or baseline vitamin D level (for change in vitamin D level), age, BMI, smoking, tibial bone area, cartilage defects, sun exposure, steps per day, season of blood sampling, asthma, cardiovascular diseases, and diabetes, and/or sex, radiographic osteoarthritis (OA), and knee pain.
‡ $P < 0.05$.

Table 5. Associations between vitamin D insufficiency and knee radiographic osteoarthritis in the medial tibiofemoral compartment at the right and left knees combined: cross-sectional data*

	Univariable OR (95% CI)	Multivariable OR (95% CI)†
JSN, grade ≥1 vs. 0	1.10 (0.85, 1.44)	1.03 (0.77, 1.38)
JSN, grade 2 and 3 vs. 0 and 1	1.82 (1.25, 2.64)‡	1.68 (1.13, 2.50)‡
Osteophytes, grade ≥1 vs. 0	1.50 (0.92, 2.42)	1.04 (0.60, 1.78)
Osteophytes, grade 2 and 3 vs. 0 and 1	2.02 (0.94, 4.33)	1.58 (0.69, 3.59)

* The dependent variable is JSN or osteophytes in the medial tibiofemoral compartment. The independent variable is vitamin D insufficiency (25[OH]D level <50 nmoles/liter vs. ≥50 nmoles/liter). JSN and osteophytes were scored on a scale of 0–3 (0 = normal and 3 = severe). OR = odds ratio; 95% CI = 95% confidence interval (see Table 1 for other definitions).
† Adjusted for age, sex, BMI, smoking, knee JSN if osteophytes, osteophytes if JSN, asthma, cardiovascular diseases, and diabetes.
‡ $P < 0.05$.

associated with a greater proportion of moderate-to-severe JSN in the medial tibiofemoral compartment at both right and left knees separately (Figure 1C) or both together (Table 5) in unadjusted and multivariable analysis. Vitamin D insufficiency was nonsignificantly associated with a greater proportion of osteophytes in the medial tibiofemoral compartment in both univariable and multivariable analyses (Table 5). Vitamin D insufficiency was not associated with JSN and osteophytes in the lateral compartment (data not shown). There were no significant associations between sun exposure (winter and summer), JSN, and osteophytes in the medial and lateral tibiofemoral compartments (data not shown).

The associations between 25(OH)D levels and knee cartilage loss all remained significant and of similar magnitude when subjects with cardiovascular disease, diabetes, or asthma were excluded from analyses (data not shown). No significant associations were documented between vitamin D insufficiency and prevalent total knee cartilage defects (OR 0.95, $P = 0.71$) or an increase in total knee cartilage defects (OR 0.97, $P = 0.91$) in multivariable analyses.

DISCUSSION

This study is the first to demonstrate, both cross-sectionally and longitudinally, positive associations between sun exposure, serum 25(OH)D levels, and knee tibial cartilage volume in older people, particularly in women and those with radiographic OA and knee pain, suggesting that vitamin D is an important hormonal contributor to cartilage homeostasis. In addition, it confirms a positive association between vitamin D insufficiency and moderate-to-severe JSN in older adults.

It has been recognized that vitamin D insufficiency in adults can result in secondary hyperparathyroidism, increased bone turnover, enhanced bone loss, and increased risk of fragility fracture (2,3); thus, vitamin D supplementation (700–800 IU/day) appears to reduce fracture risk, especially in institutionalized older people (20). In contrast, the role of vitamin D insufficiency in OA is far less understood and continues to be controversial. In part, this may be due to the use of radiographs rather than more sensitive tools such as MRI, as confirmed by the current study.

A cross-sectional study showed that, in female twins with knee OA, serum levels of 25(OH)D were associated with osteophytes but not with JSN, and the association with osteophytes disappeared after adjustment for age and BMI (9). This is similar to the cross-sectional data in the present study for osteophytes in older men and women, but we also found that serum levels of 25(OH)D were significantly associated with moderate-to-severe JSN in the medial tibiofemoral compartment at both knees before and after adjustment for possible confounding factors. We did not document any association between serum levels of 25(OH)D and JSN in the lateral tibiofemoral compartment; however, using quantitative measurement of cartilage volume, we found significant positive associations between serum 25(OH)D levels and cartilage volume at both medial and lateral tibial sites. In addition, vitamin D insufficiency was consistently negatively associated with both medial and lateral tibial cartilage volume.

Loss of cartilage volume is the hallmark of established OA, and cartilage defect development seems to be an event of early OA (21). Similar to the cross-sectional results, we found that both baseline 25(OH)D levels and change in 25(OH)D levels were positively associated with change in knee cartilage volume, and vitamin D

insufficiency predicted knee cartilage loss over 2.9 years. These findings were the most consistent at the medial tibial site and in women and those with radiographic OA and knee pain. This is concordant with a report which suggested that the expression of vitamin D receptors is greater in OA cartilage than in normal cartilage (5), suggesting that OA cartilage may be more sensitive to vitamin D. However, we did not find any association between serum levels of 25(OH)D and knee cartilage defects, which is consistent with the finding from the Boston Osteoarthritis of the Knee Study (BOKS) (8), suggesting that cartilage volume may be regulated by hormonal factors and that knee cartilage defects may be more related to nonmetabolic factors, as we have recently reported for leptin (22).

We did not obtain the radiograph at the first followup visit because radiographic measurement is insensitive to change over a relatively short period, so we cannot comment on the association between vitamin D and change in JSN in the present study. In the Framingham OA cohort study, McAlindon et al (6) reported that the risk of progression of radiographic OA over 8 years in older patients with knee OA was 3 times higher in the middle and lowest tertiles for baseline serum levels of vitamin D as compared with patients in the highest tertiles, and low vitamin D levels also predicted loss of joint space and osteophyte growth. In a longitudinal study over an 8-year period, Lane et al (7) reported that lower serum levels of vitamin D were associated with incident hip JSN but not with incident osteophytes, but they did not find any association between serum vitamin D levels and progression in radiographic hip OA. Recently, Felson et al (8) reported that in the Framingham OA offspring cohort study (over 9 years) and the BOKS (over 15 and 30 months), baseline serum vitamin D levels were not associated with loss of joint space and osteophyte growth. Factors such as study designs, samples, period, and different age groups, together with a larger relative variability in the joint space measurement than that in cartilage volume measurement (23), may explain the inconsistency of the study results assessed by radiography. Nevertheless, further MRI studies appear to be warranted.

The mechanisms of the effects of vitamin D on cartilage remain unclear. Vitamin D may have a direct effect on cartilage via vitamin D receptors, which stimulate proteoglycan synthesis by mature chondrocytes (24) and modulate the metalloproteinase activity that degrades cartilage (25). It may also affect cartilage through an effect on bone metabolism. Subchondral bone plays a pivotal role in the etiology of OA through bone remodeling (26), bone expansion (27), and increased bone turnover (28), and sufficient vitamin D in adults has protective effects on calcium metabolism, osteoblast activity, matrix ossification, and bone density, resulting in decreased bone turnover and decreased bone loss (2). Indeed, in this sample, 25(OH)D levels were positively associated with changes in hip and spine BMD over time (Ding C, et al: unpublished observations), and 25(OH)D levels were associated with reduced subchondral bone area in women, which implies a protective effect on bone as well as on cartilage.

More than 90% of the vitamin D requirement for most people comes from casual exposure to sunlight (3). Surprisingly, we found that sunlight exposure was associated with knee tibial cartilage volume, dependent only in part on vitamin D status. This most likely reflects the effect of measurement error on the assessment of both sun exposure (29) and 25(OH)D levels, but it may also suggest that sunlight exposure has an effect on cartilage through other mechanisms such as physical activity (30).

The strengths of the present study lie in MRI measurement of cartilage and the measurements of vitamin D levels on 2 occasions, which allowed us to assess cartilage loss and the variation of 25(OH)D levels over time. Our study also has several potential limitations. First, we had knee MRI scans for 880 subjects at baseline but for only 353 subjects at followup due to loss of the MRI scanner. However, there were no differences between those studied and those lost to followup, and the sample size was sufficient to detect significant associations between 25(OH)D levels and changes in cartilage volume. Second, the response rate at baseline was 57%, leaving the possibility open for selection bias. However, while the sample contained subjects with some diseases, the results were largely unchanged when the analyses were adjusted for disease status or when these subjects were excluded. We also had a high retention rate to offset this. Third, we used tibial cartilage rather than femoral cartilage as the measure of joint cartilage at the tibiofemoral joint, and it is possible that associations with femoral cartilage are different from those with tibial cartilage (31).

In conclusion, sunlight exposure and serum 25(OH)D levels are both associated with decreased knee cartilage loss (assessed by radiograph or MRI) but not with cartilage defects. This is best observed using the whole range of 25(OH)D levels rather than predefined cut points and implies that achieving vitamin D sufficiency may prevent and/or retard cartilage loss in knee OA.

ACKNOWLEDGMENTS

Special thanks go to the subjects who made this study possible. The role of C. Boon and P. Boon in collecting the data is gratefully acknowledged. We would like to thank Dr. G. Zhai, Mr. R. Warren, and Ms S. Wei for MRI readings, and Drs. V. Srikanth and H. Cooley for radiographic assessment.

AUTHOR CONTRIBUTIONS

Dr. Ding had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Ding, Cicuttini, Jones.
Acquisition of data. Ding, Parameswaran, Burgess, Jones.
Analysis and interpretation of data. Ding, Jones.
Manuscript preparation. Ding, Cicuttini, Parameswaran, Burgess, Quinn, Jones.
Statistical analysis. Ding, Quinn, Jones.

REFERENCES

1. Felson DT. Clinical practice: osteoarthritis of the knee. N Engl J Med 2006;354:841–8.
2. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266–81.
3. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 2004;80(6 Suppl):1678–88S.
4. McAlindon TE. Nutraceuticals: do they work and when should we use them? Best Pract Res Clin Rheumatol 2006;20:99–115.
5. Tetlow LC, Woolley DE. Expression of vitamin D receptors and matrix metalloproteinases in osteoarthritic cartilage and human articular chondrocytes in vitro. Osteoarthritis Cartilage 2001;9: 423–31.
6. McAlindon TE, Felson DT, Zhang Y, Hannan MT, Aliabadi P, Weissman B, et al. Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. Ann Intern Med 1996;125: 353–9.
7. Lane NE, Gore LR, Cummings SR, Hochberg MC, Scott JC, Williams EN, et al, for the Study of Osteoporotic Fractures Research Group. Serum vitamin D levels and incident changes of radiographic hip osteoarthritis: a longitudinal study. Arthritis Rheum 1999;42:854–60.
8. Felson DT, Niu J, Clancy M, Aliabadi P, Sack B, Guermazi A, et al. Low levels of vitamin D and worsening of knee osteoarthritis: results of two longitudinal studies. Arthritis Rheum 2007;56: 129–36.
9. Hunter DJ, Hart D, Snieder H, Bettica P, Swaminathan R, Spector TD. Evidence of altered bone turnover, vitamin D and calcium regulation with knee osteoarthritis in female twins. Rheumatology (Oxford) 2003;42:1311–6.
10. Cicuttini F, Forbes A, Morris K, Darling S, Bailey M, Stuckey S. Gender differences in knee cartilage volume as measured by magnetic resonance imaging. Osteoarthritis Cartilage 1999;7: 265–71.
11. Ding C, Cicuttini F, Blizzard L, Jones G. Smoking interacts with family history with regard to change in knee cartilage volume and cartilage defect development. Arthritis Rheum 2007;56:1521–8.
12. Jones G, Blizzard C, Riley MD, Parameswaran V, Greenaway TM, Dwyer T. Vitamin D levels in prepubertal children in Southern Tasmania: prevalence and determinants. Eur J Clin Nutr 1999;53: 824–9.
13. Buckland-Wright CB. Protocols for precise radio-anatomical positioning of the tibiofemoral and patellofemoral compartments of the knee. Osteoarthritis Cartilage 1995;3 Suppl A:71–80.
14. Jones G, Ding C, Scott F, Glisson M, Cicuttini F. Early radiographic osteoarthritis is associated with substantial changes in cartilage volume and tibial bone surface area in both males and females. Osteoarthritis Cartilage 2004;12:169–74.
15. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 1988;15:1833–40.
16. Jones G, Glisson M, Hynes K, Cicuttini F. Sex and site differences in cartilage development: a possible explanation for variations in knee osteoarthritis in later life. Arthritis Rheum 2000;43:2543–9.
17. Ding C, Cicuttini F, Scott F, Cooley H, Boon C, Jones G. Natural history of knee cartilage defects and factors affecting change. Arch Intern Med 2006;166:651–8.
18. Ding C, Garnero P, Cicuttini F, Scott F, Cooley H, Jones G. Knee cartilage defects: association with early radiographic osteoarthritis, decreased cartilage volume, increased joint surface area and type II collagen breakdown. Osteoarthritis Cartilage 2005;13:198–205.
19. Drape JL, Pessis E, Auleley GR, Chevrot A, Dougados M, Ayral X. Quantitative MR imaging evaluation of chondropathy in osteoarthritic knees. Radiology 1998;208:49–55.
20. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA 2005;293:2257–64.
21. Ding C, Cicuttini F, Jones G. How important is MRI for detecting early osteoarthritis? Nat Clin Pract Rheumatol 2008;4:4–5.
22. Ding C, Parameswaran V, Cicuttini F, Burgess J, Zhai G, Quinn S, et al. Association between leptin, body composition, sex and knee cartilage morphology in older adults: the Tasmanian Older Adult Cohort (TASOAC) study. Ann Rheum Dis 2008;67:1256–61.
23. Raynauld JP, Martel-Pelletier J, Berthiaume MJ, Beaudoin G, Choquette D, Haraoui B, et al. Long term evaluation of disease progression through the quantitative magnetic resonance imaging of symptomatic knee osteoarthritis patients: correlation with clinical symptoms and radiographic changes. Arthritis Res Ther 2006;8:R21.
24. Corvol MT, Dumontier MF, Tsagris L, Lang F, Bourguignon J. Cartilage and vitamin D in vitro (author's transl). Ann Endocrinol (Paris) 1981;42:482–7. In French.
25. Schmitz JP, Schwartz Z, Sylvia VL, Dean DD, Calderon F, Boyan BD. Vitamin D3 regulation of stromelysin-1 (MMP-3) in chondrocyte cultures is mediated by protein kinase C. J Cell Physiol 1996;168:570–9.
26. Burr DB. Anatomy and physiology of the mineralized tissues: role in the pathogenesis of osteoarthrosis. Osteoarthritis Cartilage 2004;12 Suppl A:S20–30.
27. Ding C, Cicuttini F, Jones G. Tibial subchondral bone size and knee cartilage defects: relevance to knee osteoarthritis. Osteoarthritis Cartilage 2007;15:479–86.
28. Bettica P, Cline G, Hart DJ, Meyer J, Spector TD. Evidence for increased bone resorption in patients with progressive knee osteoarthritis: longitudinal results from the Chingford study. Arthritis Rheum 2002;46:3178–84.
29. McCarty CA. Sunlight exposure assessment: can we accurately assess vitamin D exposure from sunlight questionnaires? Am J Clin Nutr 2008;87:1097–101S.
30. Foley S, Ding C, Cicuttini F, Jones G. Physical activity and knee structural change: a longitudinal study using MRI. Med Sci Sports Exerc 2007;39:426–34.
31. Ding C, Martel-Pelletier J, Pelletier JP, Abram F, Raynauld JP, Cicuttini F, et al. Two-year prospective longitudinal study exploring the factors associated with change in femoral cartilage volume in a cohort largely without knee radiographic osteoarthritis. Osteoarthritis Cartilage 2008;16:443–9.