MODERATE VITAMIN D DEFICIENCY IS ASSOCIATED WITH CHANGES IN KNEE AND HIP PAIN IN OLDER ADULTS: A FIVE YEAR LONGITUDINAL STUDY

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Abstract

Vitamin D is important for bone, cartilage and muscle function but there are few studies on its association with joint pain. We investigated whether serum vitamin D predicted change in knee and hip pain in older adults.

Methods: Longitudinal population-based cohort study of randomly selected older adults (n=766). Serum 25-hydroxyvitamin D (25–OHD) was assessed at baseline by radioimmunoassay and pain at baseline, 2.6 and/or 5 years using the WOMAC questionnaire. We used linear regression with adjustment for age, sex, BMI and season, then further adjusted for potential structural mechanisms (radiographic osteoarthritis, bone marrow lesions, chondral defects and muscle strength).

Results: Participants were aged 50-80 years (mean 62 years), 50% were male. Mean total knee WOMAC score was 3.2 (range 0-39). 4.2% of participants had moderate vitamin D deficiency at baseline (25–OHD 12.5 – 25 nmol/L). 25–OHD <25nmol/L predicted change in knee pain (using total WOMAC score) over 5 years (β=2.41, p=0.002) with a similar effect size for hip pain over 2.4 years (β=2.20, p=0.083). Results were consistent within pain subscales, and the association was independent of demographic, anthropometric and structural covariates. No association was present when 25–OHD was analysed as a continuous measure.

Conclusions: Moderate vitamin D deficiency independently predicts incident or worsening in knee pain over 5 years and possibly hip pain over 2.4 years. Therefore correcting moderate vitamin deficiency may attenuate worsening of knee or hip pain in elderly persons but supplementing people with a higher 25–OHD level is unlikely to be effective.

Introduction

Vitamin has vital functions in human physiology. Frank vitamin D deficiency is associated with rickets and osteomalacia[1], but less marked deficiency can also result in ill health[2, 3].

Vitamin D deficiency is common in persons with widespread bone and muscle pain[4-9] but this may be biased by reverse causation whereby illness leads to lower sun exposure. Cross sectional studies demonstrate a latitudinal gradient to joint pain[10], suggesting a role for climatic factors including vitamin D; and suggest an association between low 25–OHD and knee pain[4, 11]. Several case series suggest a possible beneficial effect of vitamin D supplementation[8, 12-15], with one exception[6]. Supplementation (500IU) reduced generalised pain after three months in patients with early rheumatoid arthritis[16], but not in two other small trials in participants with diffuse musculoskeletal pain[17, 18], or a meta-analysis in patients with a wide variety of chronic painful conditions[19], using the available low quality studies. To the best of our knowledge, there are no longitudinal cohort studies addressing this issue.

Therefore, the aim of this study was to assess the association between serum 25-hydroxyvitamin D (25–OHD) at baseline, and change in knee and hip pain as assessed by the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) questionnaire over five and 2.4 years respectively, in a cohort of randomly selected community dwelling older adults.

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PATIENTS AND METHODS

Study design, setting and participants

The Tasmanian Older Adult Cohort (TASOAC) study is a population-based cohort study, which aims to identify factors associated with the development and progression of osteoarthritis and osteoporosis in older adults. Men and women aged 50-80 years in 2002 were selected from the electoral roll in Southern Tasmania (population 229,000) using sex-stratified random sampling (response rate 57%). Participants were excluded if they lived in an aged care facility, or had contraindications to magnetic resonance imaging. The Southern Tasmanian Health and Medical Human Research Ethics Committee approved the study, and we obtained written informed consent from all participants. Baseline data (Phase 1) was collected from February 2002 to September 2004 in 1099 participants. Participants who did not have an MRI at Phase 1 (n= 105) were excluded from further participation in the study, as the primary aim of TASOAC was to measure progression of osteoarthritis. Follow up data (Phase 2 and 3) was collected on average 2.6 (range 1.4 – 4.8) and 5 years (range 3.6 – 6.9 years) later, in 875 and 768 participants respectively. Data in this paper is limited to participants with data at Phase 3.

Exposure: serum Vitamin D

Participants provided blood samples at Phase 1. Samples were treated initially with acetonitrile to rapidly extract 25–OHD. We then assayed 25–OHD using a Liquid Phase radioimmunoassay (IDS, Boldon, Tyne & Wear, UK). The intra– and interassay coefficients of variation (CVs) were 1.8% and 3.3%, respectively[20].

We used <12.5 nmol/L to define severe vitamin D deficiency, [21], 12.5–25 nmol/L

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(25–OHD) for moderate vitamin D deficiency; 25–50 nmol/L for mild deficiency, and vitamin D replete >50 nmol/L[22].

Outcomes: Knee and hip pain

Self-reported knee and hip pain for the last 30 days was assessed by questionnaire using the Western Ontario McMaster Osteoarthritis Index (WOMAC)[23] as previously described[24], with only the pain scale reported here. Briefly, the WOMAC pain scale has five items, each rated on a 10-point numeric rating scale from 0 (no pain) to 9 (most severe pain)[23]. Each pain item was summed to create a total pain (0–45) score. Knee pain was assessed at Phase 1 and 3, and hip pain at Phases 2 and 3; therefore change in knee pain is over 5 years and change in hip pain over 2.4 years. Change in WOMAC score was calculated as (follow–up value - baseline value) with difference$\geq$1 indicating worsening and $\leq$-1 improvement in knee pain.

Knee and hip radiographs

Participants had X-rays of hips (n=639) and knees (n=711) in the standing anterio–posterior (AP) position at baseline only. Knee X-rays were taken of both knees with 15° of fixed knee flexion, and pelvic radiographs with both feet in 10° internal rotation. Films were scored individually for osteophytes and joint space narrowing (JSN) each on a scale of 0–3 (0 = normal, 3 = severe) according to the Osteoarthritis Research Society International (OARSI) atlas[25]. Hips and knees with JSN or osteophyte scores ≥1 at any site were classified as having JSN or osteophytes respectively, and radiographic osteoarthritis. Two readers simultaneously assessed radiographs with immediate reference to the atlas. Scores for each participant were determined by consensus. Intraobserver repeatability was assessed in 40

participants (intraclass correlation coefficients (ICCs) 0.65 – 0.85 for knees and 0.60– 0.87 for hips)[26].

Knee bone marrow lesions and cartilage defects

Bone marrow lesions and cartilage defects of the knee were assessed on MR images of the right knee, acquired with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, OH, USA) using a commercial transmit-receive extremity coil at baseline. BMLs were assessed on T2-weighted fat saturation 2D fast spin echo MR images using Osiris software as previously described[27], and were defined as areas of increased signal adjacent to the subcortical bone at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites. BMLs were dichotomised as being present (BML area >0 mm) or absent (BML area = 0 mm).

Cartilage defects were assessed by a trained observer on T1-weighted fat saturation 3D spoiled gradient recalled MR images (score range, 0 – 4) at the tibial and femoral sites, medially and laterally, as previously described[28] as follows: grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface and base; grade 2 = irregularities on the surface or base and loss of thickness < 50%; grade 3 = deep ulceration with loss of thickness > 50%; and grade 4 = full-thickness chondral wear with exposure of subchondral bone. A cartilage defect had to be present on at least 2 consecutive slices. The cartilage was considered to be normal if the band of intermediate signal intensity had a uniform thickness. If >1 defect was present in the same site the highest grade was used.

Other factors

Leg strength was measured to the nearest kilogram in both legs simultaneously, using a dynamometer (TTM Muscular Meter, Tokyo, Japan) as previously

described[29]. BMI was calculated [weight (in kilograms)/height (in meters)^2] using weight measured to the nearest 0.1 kg (with shoes, socks, bulky clothing and headwear removed) using a single pair of calibrated electronic scales (Seca Delta Model 707), and height measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. Alcohol intake was assessed by a validated dietary questionnaire (The Cancer Council Victoria, Victoria, Australia)[30] as previously described[31], and measured in glasses per day.

Statistical methods

We used Stata 12.0 (StataCorp LP) for statistical analyses. Statistical significance was set as a p value ≤0.05 (two-tailed). Differences in sample characteristics between participants whose pain worsened by ≥1 unit and those in whom pain was unchanged or improved were assessed using students' t–tests or χ^2 tests. The association between 25–OHD and change in WOMAC pain scale was assessed using linear regression; first with 25–OHD as a continuous measure, then as a categorical measure. As a threshold effect was detected at 25 nmol/L (data not shown), data was dichotomised at this level. Models were adjusted first for age, sex, BMI and season (summer–autumn vs winter–spring) and then additionally adjusted for structural factors and factors associated with knee pain[27], or hip pain, as appropriate. Change scores were normally distributed, although leptokurtic due to a large number of participants without pain at both baseline and follow up. Nevertheless, we reported results with robust standard errors to accommodate mild violations of homoskedasticity and normality of residuals.

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Results

Participants

764 participants had complete data for change in knee WOMAC score, and 765 for change in hip WOMAC score as well as baseline 25–OHD. Participants who did not complete Phase 3 were older, had higher BMI, lower serum 25–OHD levels and worse total knee WOMAC scores at baseline than those who remained in the study (Table 1).

Descriptive data

The prevalence of knee pain (knee WOMAC score >0) was 53% (n=582) at Phase 1 and 45% (n=346) at Phase 3. Mean change in total WOMAC score over five years among participants who experienced incident or worsening knee pain was 4.6±4.7 (n=175, range 1-24). These participants had higher BMI, weaker leg strength, more cartilage defects, were more likely to have radiographic knee OA (including osteophytes), more likely to use pain medicines (all p≤0.05), and a trend to higher prevalence of knee BMLs (p=0.11) than participants whose pain remained static or improved (Table 2).

Prevalence of hip pain at Phase 2 was 35% (n=272) and 37% at Phase 3 (n=191), with mean change in total WOMAC score of 5.7±6.6 (n=187; range 1-40) amongst participants who experienced incident or worsening hip pain between Phases 2 and 3. These participants had higher BMI, weaker leg strength, were more likely to use pain medicines at Phase 1 (p≤0.05), and had a trend to older age than participants whose pain remained static or improved (p=0.08) (Table 2).

Mean baseline Vitamin D was 54 nmol/L (95% CI 52.5 – 55.2; range 13-116). 4.2% of participants (n=32) had moderate deficiency (25-OHD 12.5-25 nmo/L). None had severe deficiency (<12.5nmol/L).

Proportion of participants reporting new or worsening pain

The pattern of the proportion of participants with incident or worsening pain being greater in participants with lower 25–OHD (Figure 1a, Figure 1b), was consistent across subscales at both the knee and hip. Differences were statistically significant for the total knee WOMAC score and subscales of "going up and down stairs", "sitting or lying down" and "standing upright". For the hip, the effects were consistent in direction, but were only statistically significant for one scale ("pain whilst climbing stairs"). Differences in the proportion of participants reporting incident and worsening pain for total WOMAC hip pain and the remaining 4 subscales among 3 categories of 25–OHD were not significant (Figure 1b).

Change in knee and hip pain

Participants with serum 25–OHD 12.5 – 25 nmol/L (moderate vitamin D deficiency) experienced greater worsening of total knee WOMAC pain score over five years, than participants with 25–OHD above this level (Figure 2). Associations with total hip WOMAC pain over 2.4 years did not reach statistical significance. Any relationship between serum 25–OHD and change in knee and hip WOMAC scores was not linear (Table 3, Table 4). When data were dichotomised at 25 nmol/L, having 25–OHD <25 nmol/L predicted incident or worsening pain in total knee WOMAC score and two of the five subscales over 5 years. Effect sizes remained unchanged or strengthened after adjustment for baseline covariates.

Similar patterns were present when change in hip pain was the outcome, over a shorter period of observation but coefficients were slightly smaller. Associations did not reach statistical significance in univariate or multivariate models (see Table 4).

Sensitivity analyses

Participants often had baseline and follow up interviews in different seasons, which could affect results. We therefore repeated analyses for the 18% (n=137) of participants who had their initial and follow-up interviews in the same season. Using multivariate model 1 (Table 3, Table 4), effect sizes increased in magnitude for both change in knee pain and change in hip pain: knees β=3.99 (95% CI 0.52 – 7.44; p=0.024), and hips β=3.72 (95% CI -0.52 – 7.97; p=0.085).

As other authors propose an alternate definition of moderate deficiency (30 nmol/L)[32], we conducted sensitivity analyses around the threshold of vitamin D for dichotomous models, using a cutoff of 30 nmol/L, and multivariate model 1 (Table 3, Table 4). Associations reduced in size but remained significant for change in knee pain, from β=2.41 (95% CI 0.85 – 3.96; p=0.002) to β=1.90 (95% CI 0.94 – 2.87; p<0.001). For change in hip pain, effect size reduces from β=2.20 (95% CI -0.29 – 4.69; p=0.083), to β=0.91 (95% CI -0.38 – 2.21; p=0.17) when 30 nmol/L was used as the cutoff.

Discussion

To the best of our knowledge, this study is the first to show that moderate vitamin D deficiency predicts change in knee pain, in a cohort of community dwelling older adults. This effect was evident only in participants with 25–OHD ≤25 nmol/L, consistent with 25–OHD levels where PTH levels become more markedly elevated[32] and osteomalacia becomes increasingly common[21].

This study is also the first to report on potential associations between 25–OHD and change in hip pain; effect sizes are of a similar magnitude and the same direction to those observed in the knee, though they did not reach statistical significance. While we cannot rule out that the lack of significance in associations between 25–OHD and hip pain indicates the lack of a true effect, the consistent pattern and direction of effect across pain subscales, and anatomical sites suggest that there may be an effect of moderate vitamin D deficiency on hip pain which we lacked the power to detect. Hip data was available over a shorter period of observation, compared to the knee, potentially resulting in smaller effect sizes, and larger standard errors. These contributed to our inability to detect an effect should it be present. Replicating this analysis in either a larger sample or with a longer period of follow-up is needed to clarify this.

Our results provide an explanation for the ecological findings of a latitudinal gradient and joint pain[10], and the threshold effect is consistent with cross–sectional associations[11, 33], where the lowest tertile of 25–OHD (17-35.8 nmol/L) was associated with knee pain (OR 1.47, p=0.08), but the middle tertile was not (35.9 – 51 nmol/L; OR 1.04, p=0.83)[11]. Hirani[33] reported significant associations

between 25–OHD < 75 nmol/L and generalised pain, but the strength of associations diminished markedly with higher 25–OHD[33].

We used baseline 25–OHD from one occasion, but since 25–OHD levels track over time[34-36], this is a reasonable measure of usual 25–OHD status.

As knee pain is episodic, measuring change in knee pain between two time points over five years may miss real change during this period, thereby misclassifying pain status and diluting effect sizes. However, obesity measures[37], and inflammatory markers[24] predicted change in knee pain in this cohort, in addition to low 25–OHD, suggesting that this is acceptable method.

There is moderate evidence suggesting that low 25–OHD may be positively associated with progression of radiographic OA[38], but the association between 25–OHD and pain in our cohort was largely independent of other factors[27] as adjustment for these did not reduce the size of the beta coefficients and even increased them. Therefore, in our cohort, structural factors did not confound associations between 25–OHD and pain.

Low 25–OHD is associated with muscle weakness[39], and dose–response associations between serum 25–OHD and appendicular muscle mass have been reported in our sample[40], but the association between low serum 25–OHD and joint pain in our sample persisted after adjusting for leg muscle strength. Numerous studies have investigated associations between pain and structural features of knee and hip OA. However, adjusting for structural features of OA (eg osteophytes, JSN, cartilage defects) in our study did not decrease the association between low 25–OHD and knee pain on most subscales. Overall, this suggests the mechanism of the

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relationship between vitamin D and pain may be independent of structural features of osteoarthritis.

Plausible mechanisms of 25–OHD–mediated pain include synovial inflammation, osteomalacia, or hyperparathyroidism. The active metabolite $1,25(OH)_2D$ has an antiproliferative effect and down-regulates inflammatory markers[41], which are associated with change in non–weight bearing knee pain[24]. Impaired bone mineralisation (from secondary hyperparathyroidism) allows the osteoid matrix to absorb fluid and expand, causing outward pressure on the innervated periosteal tissues, causing pain[42]. Unfortunately, assays for parathyroid hormone were not available in this cohort.

There is ongoing debate as to the level of vitamin D at which a person is moderately deficient. Some authors (including us) used 25 nmol/L[22, 43, 44], — the accepted definition at the time of this study[43]; others used 30 nmol/L[32, 45]. Sources of discrepancies include measurement error, use of different vitamin D assays, and choice of outcome measure. In our sample, increasing the cutoff to 30 nmol/L reduced effect sizes for knee pain, but the association between low serum 25–OHD and incident and worsening knee pain remained statistically significant. However, associations between 25–OHD and incident and worsening hip pain were no longer evident, suggesting that a lower threshold of 25 nmol/L may be more appropriate for pain outcomes.

Strengths of our study include its longitudinal design and the community dwelling cohort, therefore our findings are more readily generalisable to community dwelling older adults.

Limitations of our study include the limited range of sites of data on pain severity (hips and knees), short duration of followup for hip pain, and differential loss of follow up in our sample — older participants who had worse pain and lower 25–OHD at baseline preferentially dropped out; potentially biasing the results. However, as the relationship was strongest in those with low serum 25–OHD, it is likely that our findings may underestimate the strength of the associations. The absence of participants with severe deficiency (25–OHD <12.5 nmol/L) prevents us from exploring associations between severe vitamin D deficiency and pain. We did not perform frequent 25–OHD measures; therefore the duration of vitamin D deficiency required before pain increases is unknown.

Lastly, this is an observational study. Prospective trials are required to assess whether vitamin D supplementation is effective in preventing or reducing intensity of joint pain, especially at lower levels of serum 25–OHD.

In conclusion, moderate vitamin D deficiency independently predicts change in knee pain over 5 years and possibly hip pain over 2.4 years. Therefore correcting moderate vitamin deficiency may attenuate worsening of knee or hip pain in elderly persons but supplementing people with higher 25–OHD levels is unlikely to be effective.

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Authors roles

GJ designed and obtained funding for the original TASOAC study. JB and VP contributed expertise and laboratory support for analysis of serum samples for vitamin D metabolites. Analyses were designed by CD LLL and GJ, and conducted by LLL with advice from SJQ. LLL SJQ TMW GJ and CD contributed to data interpretation. All authors drafted the article and critically revised it for important intellectual content, and approved the final version of the article.

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Tables

Table 1: Characteristics of study cohort at baseline in participants who did and did not complete Phase 3

	Completed Phase 3 Mean (sd) n=769	Did not complete Phase 3 Mean (sd) n=330	p
Vitamin D (nmol/L)	53.9 (18.9)	49.0 (17.7)	**0.0001**
Proportion of patients with Vitamin D <25 nmol/L (%)	4.2	6.1	0.16
Age (years)	62.1 (7.0)	65.2 (8.1)	**<0.001**
Sex (% female)	50.5	45.2	0.11
Body mass index	27.7 (4.6)	28.4 (5.1)	**0.02**
Total knee WOMAC score	3.3 (5.6)	5.0 (7.8)	**<0.001**

Bolded results indicate statistically significant difference at $\alpha=0.05$

Table 2: Characteristics of study cohort at baseline

	Knee pain			Hip pain		
	Improved or unchanged[§]	New or worsening		Improved or unchanged[§]	New or worsening	
	Mean (SD) n= 591	Mean (SD) n= 175	p	Mean (SD) n= 580	Mean (SD) n= 187	p
Age	62.0 (7.0)	62.4 (7.1)	0.48	61.8 (6.8)	62.9 (7.7)	0.08
Gender (% male)	52	46	0.16	51	49	0.60
BMI	**27.3 (4.3)**	**29.1 (5.3)**	**<0.001**	**27.4 (4.4)**	**28.4 (5)**	**0.01**
Current smokers (%)	11	11	0.78	10	13	0.31
Alcohol intake (g/day)	2.7 (2.2)	2.5(1.9)	0.26	2.6 (2.1)	2.7 (2.3)	0.65
Leg strength (kg)	**97.6 (50)**	**88.4 (47.5)**	**0.03**	97.1 (49.1)	90 (50.7)	0.11
Use of pain meds (%)	**52**	**66**	**<0.001**	**53**	**64**	**0.01**
Baseline WOMAC knee pain score	3.4 (5.9)	3.0 (4.9)	0.42			
Phase 2 WOMAC hip pain score				2.2 (5.2)	2.8 (5.3)	0.13
Knee osteophyte (%)	**8.6**	**26.4**	**<0.001**	-	-	-
Knee ROA (%)	**62**	**74**	**0.01**	-	-	-
Any BML (%)	33	66	0.11	-	-	-
Cartilage defects	4.0 (1.6)	4.6 (2.0)	**0.0001**	-	-	-
Hip JSN (%)	33	32	0.81	32	33	0.84
Hip ROA (%)	-	-	-	43	46	0.45
Baseline 25–OHD result (nmol/L)	54.5 (18.6)	51.7 (19.9)	0.08	54.3 (18.7)	52.4 (19.5)	0.23

[§]Includes participants with no pain at both time points

Bolded results indicate statistical significance at α=0.05

All data presented is from baseline (Phase 1) unless otherwise indicated.

Table 3: Association between serum 25–OHD and change in knee pain over 5 years, as assessed by the WOMAC questionnaire

	Univariate Beta coefficient (95% CI) n=764	p	Multivariate model 1 Beta coefficient (95% CI) n=764	p	Multivariate model 2 Beta coefficient (95% CI) n=325	p
Vitamin D (continuous measure)						
Change in total knee WOMAC score	-0.01 (-0.03 to 0.01)	0.27	-0.01 (-0.03 to 0.01)	0.37	-0.02 (-0.05 to 0.02)	0.35
Change in knee pain when….						
Walking on a flat surface	-0.0005 (-0.005 to 0.004)	0.83	-0.0033 (-0.01 to 0.003)	0.34	-0.005 (-0.015 to 0.005)	0.33
Going up and down stairs	-0.003 (-0.009 to 0.003)	0.32	-0.001 (-0.007 to 0.006)	0.78	-0.001 (-0.011 to 0.008)	0.77
At night while in bed	-0.003 (-0.009 to 0.003)	0.28	-0.002 (-0.006 to 0.003)	0.45	0.001 (-0.006 to 0.008)	0.85
Sitting or lying	-0.003 (-0.007 to 0.001)	0.19	-0.003 (-0.007 to 0.002)	0.28	-0.006 (-0.012 to 0.001)	0.09
Standing upright	-0.001 (-0.006 to 0.003)	0.57	-0.01 (-0.03 to 0.01)	0.37	-0.02 (-0.05 to 0.02)	0.35
Vitamin D (<25 nmol/L compared to ≥25 nmol/L)						
Change in total knee WOMAC score	**2.41 (0.88 to 3.94)**	**0.002**	**2.41 (0.86 to 3.96)**	**0.002**	**2.96 (0.54 to 5.37)**	**0.017**
Change in knee pain when….						
Walking on a flat surface	0.32 (-0.25 to 0.89)	0.27	0.35 (-0.23 to 0.93)	0.24	0.09 (-0.58 to 0.76)	0.78
Going up and down stairs	**0.70 (0.18 to 1.23)**	**0.009**	**0.72 (0.17 to 1.27)**	**0.01**	0.87 (-0.11 to 1.85)	0.081
At night while in bed	**0.37 (0.06 to 0.68)**	**0.019**	0.29 (-0.01 to 0.6)	0.061	**0.56 (0.05 to 1.06)**	**0.032**
Sitting or lying	**0.48 (0.17 to 0.79)**	**0.002**	**0.46 (0.16 to 0.76)**	**0.003**	**0.61 (0.08 to 1.14)**	**0.024**
Standing upright	0.55 (-0.02 to 1.12)	0.059	**0.60 (0.04 to 1.16)**	**0.035**	0.89 (-0.09 to 1.87)	0.076

Bolded results indicate statistical significance at α=0.05
Model 1: Adjusted for age, sex, BMI and season (summer, autumn / winter, spring)
Model 2: Further adjusted for leg strength, hip joint space narrowing, osteophytes, number of cartilage defects, presence of knee bone marrow lesions and use of pain medications at baseline (these were only available in 49% at baseline). Hip JSN was used as a covariate as hip JSN is a correlate of knee pain while knee JSN is not[27]
Change in WOMAC score is from Phase 1 to Phase 3 (5 years).

Table 4: Association between serum 25–OHD and change in hip pain over 2.4 years, as assessed by the WOMAC questionnaire

	Univariate		Multivariate model 1		Multivariate model 2	
	Beta coefficient (95% CI) n=765	p	Beta coefficient (95% CI) n=765	p	Beta coefficient (95% CI) n=602	p
Vitamin D (continuous measure)						
Change in total hip WOMAC score	-0.002 (-0.02 to 0.02)	0.83	0.0003 (-0.02 to 0.02)	0.97	0.01 (-0.01 to 0.04)	0.27
Change in hip pain when….						
Walking on a flat surface	-0.001 (-0.006 to 0.004)	0.70	-0.001 (-0.006 to 0.004)	0.82	0.002 (-0.004 to 0.007)	0.58
Going up and down stairs	-0.001 (-0.006 to 0.004)	0.61	0.0006 (-0.004 to 0.006)	0.81	0.003 (-0.003 to 0.008)	0.31
At night while in bed	0.0001 (-0.005 to 0.005)	0.98	0.0002 (-0.005 to 0.006)	0.95	0.003 (-0.003 to 0.009)	0.33
Sitting or lying	0.001 (-0.004 to 0.005)	0.80	0.001 (-0.004 to 0.005)	0.70	0.004 (-0.001 to 0.009)	0.11
Standing upright	-0.001 (-0.005 to 0.004)	0.80	-0.001 (-0.005 to 0.004)	0.76	0.002 (-0.003 to 0.007)	0.42
Vitamin D (<25 nmol/L compared to ≥25 nmol/L)						
Change in total hip WOMAC score	2.19 (-0.26 to 4.64)	0.08	2.20 (-0.29 to 4.69)	0.083	2.31 (-0.69 to 5.3)	0.13
Change in hip pain when….						
Walking on a flat surface	0.42 (-0.26 to 1.10)	0.22	0.42 (-0.27 to 1.11)	0.23	0.54 (-0.29 to 1.38)	0.20
Going up and down stairs	0.52 (-0.05 to 1.09)	0.07	0.48 (-0.09 to 1.05)	0.09	0.52 (-0.17 to 1.21)	0.14
At night while in bed	0.48 (-0.13 to 1.09)	0.12	0.50 (-0.12 to 1.12)	0.12	0.52 (-0.14 to 1.19)	0.12
Sitting or lying	0.30 (-0.03 to 0.63)	0.07	0.31 (-0.05 to 0.66)	0.09	0.17 (-0.23 to 0.57)	0.40
Standing upright	0.47 (-0.16 to 1.10)	0.14	0.49 (-0.13 to 1.11)	0.12	0.47 (-0.3 to 1.25)	0.23

Bolded results indicate statistical significance at $\alpha=0.05$
Model 1: Adjusted for age, sex BMI and season (summer, autumn / winter, spring)
Model 2: Further adjusted for leg strength, radiographic hip OA (present / absent), use of pain medications at baseline, and baseline WOMAC score (same scale eg baseline total hip WOMAC score for change in total hip WOMAC score).
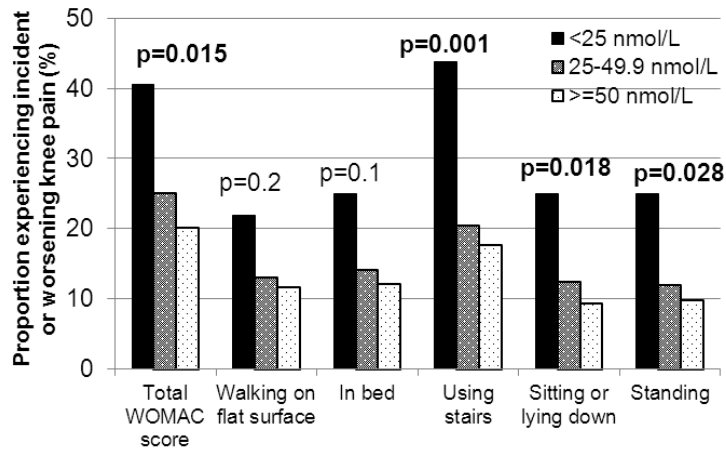Change in WOMAC score is from Phase 2 to Phase 3 (2.4 years).

Figure 1a: Proportion of TASOAC participants reporting incident or worsening knee pain on the WOMAC total pain scale and subscales over 5 years of observation, by category of baseline serum 25–OHD (p values using χ^2 tests)
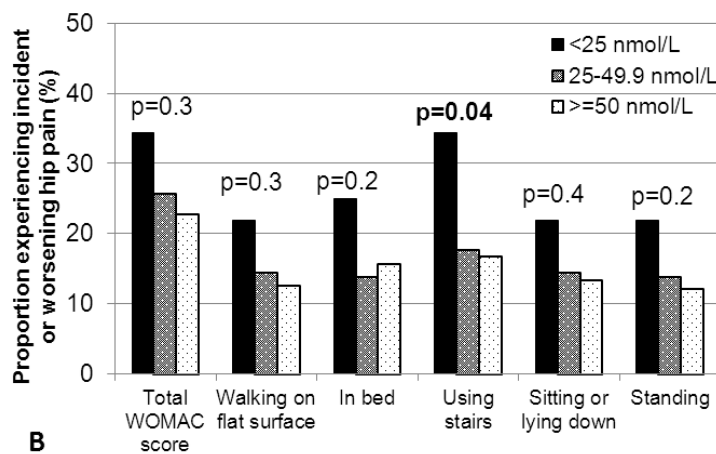

Figure 1b: Proportion of TASOAC participants reporting incident or worsening hip pain on the WOMAC total pain scale and subscales over 2.4 years of observation, by category of baseline serum 25–OHD (p values using χ^2 tests)
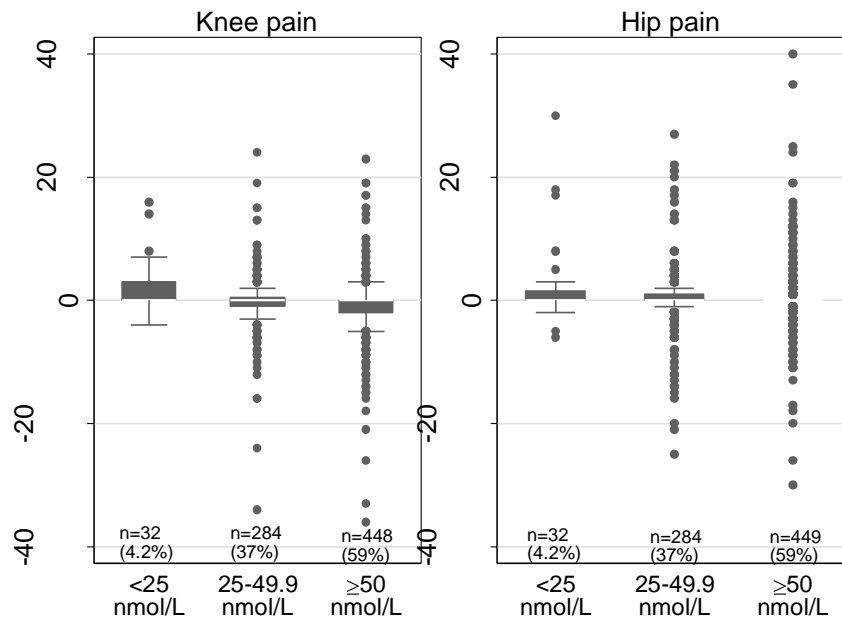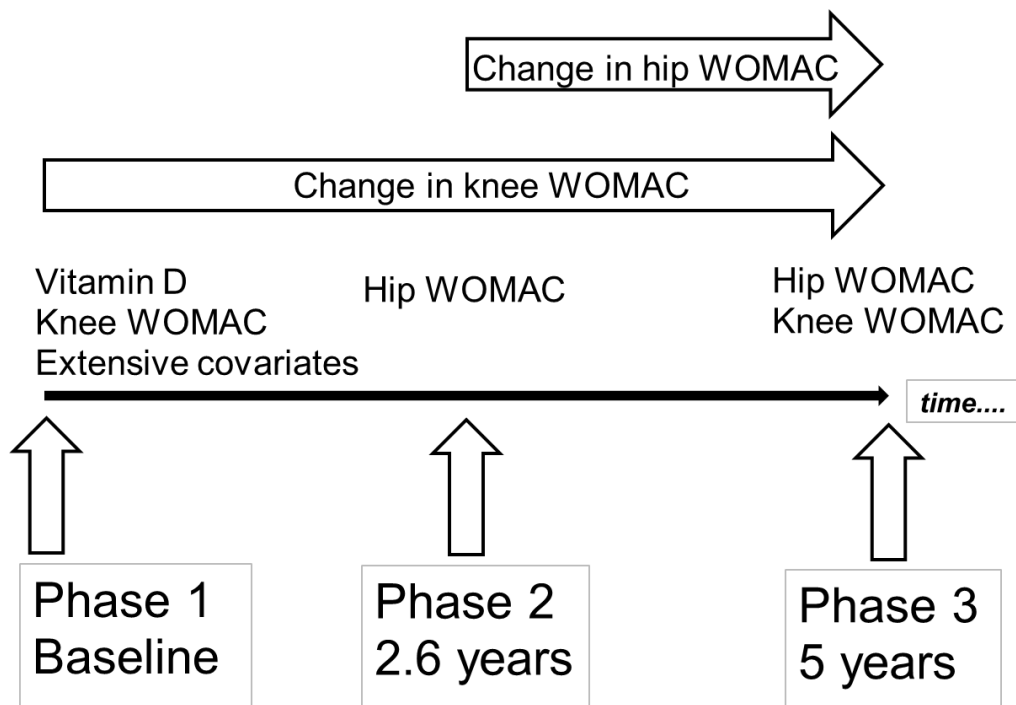
Figure 2: Boxplot of change in total knee WOMAC pain score over 5 years and change in total hip WOMAC pain score over 2.4 years, by categories of 25–OHD (nmol/L) at baseline. Knee pain: p for trend 0.002, threshold model (above / below 25 nmol/L) p=0.009. Hip pain: p for trend 0.63, threshold model p=0.026. Note: Interquartile range of change in hip WOMAC pain score in participants with 25–OHD of ≥50 nmol/L is 0 to 0.

Supplemental figure: Study flow chart indicating the nature and timing of data collection

Table and figure legends

Table 1: Characteristics of study cohort at baseline in participants who did and did not complete Phase 3

Table 2: Characteristics of study cohort at baseline

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Figure 1b: Proportion of TASOAC participants reporting incident or worsening hip pain on the WOMAC total pain scale and subscales over 2.4 years of observation, by category of baseline serum 25-OHD (p values using χ^2 tests)

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