**Author's response to reviews**

**Title:** Vitamin D supplementation in the management of knee osteoarthritis: study protocol for a randomized controlled trial

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**Author's response to reviews:** see over
6 June 2012

Editor-in-Chief, Trials
University of Oxford, United Kingdom

Dear managing editor,

We are very grateful to receive the reviewer’s comments on our manuscript. We have carefully considered these comments and have revised the manuscript accordingly as outlined below:

1) Sample size: Could you discuss this more fully and be more transparent, and, in particular, (i) give the standard deviation used in the calculation and its source, (ii) relate your 2.2% medial tibial cartilage volume loss to your previous findings (A&R 2009;60:1381) of a 2.1% volume loss in vitamin D sufficient patients and 2.9% in Vitamin D insufficient patients, and (iii) relate your findings to those of Losina (Abstract 385, Osteoarthritis and Cartilage 19S1, 2011, page S177) of a medial femorotibial compartment cartilage volume loss of 6.7% over 2 years?

Sample size calculations
All sample size calculations assume $\alpha=0.05$ and $\beta=0.20$ and are performed based upon formulae provided by Cohen (*Cohen J. Statistical power analysis for the behavioral sciences. Second ed. New Jersey: Lawrence Erlbaum Associates, Inc., Publishers; 1988*). Table 2 describes the sample size (each arm) needed to detect the specified differences between the placebo and vitamin D arms with at least 80% power for each outcome.

Previous studies, including our own, suggest that OA patients have a loss of cartilage volume of 4-5% per year at different joint sites, respectively (*Wluka AE, Stuckey S, Snaddon J, Cicuttini FM. The determinants of change in tibial cartilage volume in osteoarthritic knees. Arthritis Rheum 2002;46:2065-72*). Vitamin D supplementation in doses ranging from 400 to 800 IU/d increases serum level of 25-(OH)D by 27 nmol/liter per year in 7964 men and women from 5 studies (*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*). We estimate from our published data (*Ding C, Cicuttini F, Parameswaran V, Burgess J, Quinn S, Jones G: Serum levels of vitamin D, sunlight exposure, and knee cartilage loss in older adults: the Tasmanian older adult cohort study. Arthritis Rheum 2009, 60:1381-89*) that this change will lead to absolute reductions in loss of cartilage volume by 2.2% at medial tibial site after vitamin D supplementation. The sample size that is needed to detect this difference is calculated (Table 2).

We have shown that OA patients had an increase in medial tibial bone area in men of $1.6 \pm 2.8\%$ per year (*Wang YY, Wluka A, Cicuttini F. The determinants of change in...*)
tibial plateau bone area in osteoarthritic knees: a cohort study. Arthritis Research & Therapy; 7: 687-693, 2005), and an incidence of knee cartilage defects of 80% over 2 years (Wluka A, Ding C, Jones G, Cicuttini F. The clinical correlates of articular cartilage defects in symptomatic knee osteoarthritis: a prospective study. Rheumatology 2005; 44:1311-6). There is no data known to the investigators about the associations between change in vitamin D and change in bone area or cartilage defects. However, healthy subjects had an increase in tibial bone area of 0.7% per year (Ding et al, unpublished), and an incidence of knee cartilage defects of 65% over 2 years in older people (Wang Y, Ding C, Wluka A, Davis S, Ebeling P, Jones G, Cicuttini F. Factors affecting progression of knee cartilage defects in normal subjects over 2 years. Rheumatology 2006; 45: 79-84). Assuming that changes in cartilage defects and bone area in OA patients will be suppressed by vitamin D supplementation to the levels in the healthy subjects, the sample size needed to detect these differences is given in Table 2.

Therefore, 200 patients in each arm (allowing for a 20% dropout over the trial) will be sufficient to detect the differences between treatment groups.

Table 2. Sample size calculation

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Detectable difference</th>
<th>Calculated sample size (per arm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of medial tibial cartilage volume</td>
<td>4.5% ± 6.5%</td>
<td>2.16%</td>
<td>143</td>
</tr>
<tr>
<td>Increase in medial tibial bone area</td>
<td>1.6 ± 2.8%</td>
<td>0.9%</td>
<td>153</td>
</tr>
<tr>
<td>Incidence of knee cartilage defects</td>
<td>80%</td>
<td>15%</td>
<td>136</td>
</tr>
</tbody>
</table>

Now we have replaced original descriptions of sample size calculation by these (page 14-15).

In terms of our previous findings in our A&R paper, these data are unadjusted and were from older community-based adults so we did not use them for sample size calculation in our current study targeting at OA population. The reviewer also mentioned Losina’s report on medial femorotibial cartilage volume loss (6.7% over 2 years, approximately 4% annually). It was recently reported and the rate of loss is comparable to that for medial TIBIAL cartilage we used in this study.

2) Analysis plan:

Could you unambiguously specify the primary analysis: linear regression, logistic regression, covariates, adjustments, method of dealing with missing data, etc.?
Consider a secondary analysis of cartilage volume using a binary measure based on MCID where “success” is “progression less than cartilage volume MCID” and “failure” is “progression more than the cartilage volume MCID”.

We have added following descriptions to the text (page 16-17):

The independent t-tests will be used to compare changes between groups in quantitative data from baseline to the end of follow-up. Linear regression (annual changes in cartilage volume, cartilage defects and muscle strength as the dependent variables, treatments as the independent variable) and logistic regression (development/progression of bone marrow lesions and meniscal abnormalities as the dependent variables, treatments as the independent variable) analyses will be applied in univariable and multivariable modelling adjusted for age, sex, body mass index, baseline 25-(OH)D and other disease status.

In secondary analysis of cartilage volume loss, the minimal clinically important differences (MCID) in cartilage volume will be calculated (ref Losina’s paper mentioned by reviewer) and logistic regression will be used to determine the association between cartilage loss (>=MCID vs <MCID) and treatments before and after adjustment for above covariates.

The last-observation-carried forward method will be used in the analysis of all outcomes among patients who made at least one follow-up visit but did not complete whole.

3) Overall interpretation:

I assume the safety profile of vitamin D will not be problematic. Nevertheless, a statistically significant but quantitatively modest lessening of cartilage volume loss will be difficult for clinicians to interpret unless the WOMAC result is strongly positive. If volume loss is only modest and there is no dramatic WOMAC gain, is long term treatment with an uncertain benefit worthwhile? The point here is that cartilage volume loss has not been established as a valid surrogate for, i.e., predictive for, say, progression to joint replacement, and past reliance on observational data alone to support surrogacy has often led to erroneous conclusions. Interventional studies targeting both the putative surrogate marker, volume loss, and some patient important clinical outcome are needed to establish valid surrogacy.

In light of this, consider making cartilage volume and the WOMAC co-primary endpoints, and pre-specify that a successful trial needs success in both co-primary endpoints. At a minimum, assuming the trial is well underway but no patient is yet finished two years, you might add an exit (2-year) semi-flexed knee-x-ray to enable the dataset to be studied along with others that have measured X-ray change over two years.
In the same vein, the language in the summary paragraph seems a overstated. In particular, the sentence: “If correcting vitamin D deficiency can reduce rates of cartilage loss to lower levels as seen in older people without OA, it will significantly prolong the time it takes to reach end-stage OS eventually requiring joint replacement.” This is only true if cartilage volume slowing associated with a therapy predicts a reduction in time to or number of joint replacements associated with therapy, a claim to date not substantiated.

We would like to thank the reviewer’s overall interpretation. We have expected that vitamin D supplementation will have disease-modifying effects on knee OA based on our previous findings (A&R 2009) but agree that symptom control and disease modification should be equally important for an OA therapeutic agent. Indeed, we recently reported that baseline lower serum 25-(OH)D levels were associated with increased incidence of knee pain over 5 years (Laslett L, Ding C, Quinn S, Burgess J, Parameswaran V, Winzenberg T, et al; Serum 25 Hydroxy Vitamin D (25OHD) and Incident or Worsening Knee Pain In Older Adults: A Five Year Longitudinal Study. [abstract]. Arthritis Rheum 2011; 63 Suppl 10 :876) suggesting that vitamin D supplementation may also have a symptom-relieving effect. Now we have listed cartilage volume change and WOMAC as co-primary endpoints (we have made changes throughout the paper).

However, we have different opinion about the comment on cartilage volume. Our structural outcome measures (including loss of cartilage volume, cartilage defects, tibial bone area, bone marrow lesions) all were predictive of total knee replacement, independent of potential confounders (This has been added to Discussion, page 18) (Cicuttini FM, Jones G, Forbes A, Wluka AE. Rate of cartilage loss at two years predicts subsequent total knee arthroplasty: a prospective study. Ann RheumDis 2004;63(9):1124-7; Wluka AE, Ding C, Jones G, Cicuttini FM. The clinical correlates of articular cartilage defects in symptomatic knee osteoarthritis: a prospective study. Rheumatology (Oxford) 2005;44(10):1311-6; Raynauld JP, Martel-Pelletier J, Haraoui B, Choquette D, Dorais M, Wildi LM, Abram F, Pelletier JP: Risk factors predictive of joint replacement in a 2-year multicentre clinical trial in knee osteoarthritis using MRI: results from over 6 years of observation. Ann Rheum Dis 2011, 70:1382-8). Therefore, the knee structural measures (including cartilage volume) are clinically relevant and valid, and the statement in the summary paragraph seems not be overstated.

We have opted not to do x-ray at 2 years’ follow up because it lacks sensitivity for changed over 2 years (this was mentioned in page 18).

One minor suggestion:

P10/Cartilage column: The second sentence regarding resampling should be explained in language the does not require the reader to dig back into multiple past reports to have some understanding of what you’re doing.
We have added more descriptions on resampling of cartilage volume measurement (page 10).

We have also added a sentence to introduce the informed consent at the Editor’s request (Informed written consents will be obtained from all participants, page 7).

We made all revisions in track-change version of the manuscript with added references highlighted in yellow. We also provided a final clear version of the manuscript as well.

We look forward to hearing from you.

Yours sincerely,

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