Author's response to reviews

Title: The effects of vitamin D2 or D3 supplementation on glycaemic control and related metabolic parameters in people at risk of type 2 diabetes: protocol of a randomised double-blind placebo-controlled multi-centre trial

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Author's response to reviews: see over
Dear Editors,

Re-submission of MS: 1717493680836992: “The effects of vitamin D$_2$ or D$_3$ supplementation on glycaemic control and related metabolic parameters in people at risk of type 2 diabetes: protocol of a randomised double-blind placebo-controlled trial”

Thank you for the consideration of our manuscript and the opportunity to address the concerns of the reviewers. We have responded to each of the points raised by the reviewers and are pleased to submit a revised version. We believe these revisions have improved the paper and we are grateful to the reviewers for their input.

We look forward to the consideration of our re-submission, and we believe that this paper should be of great interest to the readership of BMC Public Health as the topic of the potential role of vitamin D in chronic disease remains an important one.

Yours sincerely

Dr Nita Forouhi, Dr Ravi Menon and Professor Graham Hitman

On behalf of all co-authors

08/10/2013
Reviewer 1:

Comment: Menon and colleagues have designed a very comprehensive trial on the efficacy of two vitamin D isoforms at high dosages for 4 months, with circulating 25(OH)D and HbA1c as the primary outcome and several other biochemical variables as the secondary outcome. The study is well designed and using HPLC/MS for 25(OH)D determination further adds to the validity of the findings. However, there are only some very minor and a few major points:

Response: Thank you for the acknowledgement of the comprehensive and well-designed nature of our work. We are pleased to address the comments as below.

Minor issues:

1- Abstract, Methods, last sentence would be better if it were “participants are recruited”, though may have recruited your participants already!

Response: Done – please see abstract.

2- P7: Please change citations from [23-24][25-27] to [23-27].

Response: Done.

3- P16: I didn’t get that if blood samples taken on the second and third visits are analyzed at the same time or they are evaluated at the end of the intervention. It is stated that all variables, except for A1c (and probably FSG!), will be tested finally. However, “safety analysis” as a monitoring approach is expected to be done at the time of bleeding. Please make more clarification.

Response: We have now made this clearer on P16 (lines 5-11). We are using point of care measurement of ionised calcium for safety assessment; hence bloods are checked during the visit and further doses of the vitamin D/placebo would only be administered once the results are available and within the safety parameters. To ensure accuracy of point of care assessment, we are also sending the bloods to the laboratory for serum calcium assessment. The laboratory results are checked prior to the next patient visit. Only safety bloods i.e. serum calcium is checked contemporaneously, other bloods are stored and send evaluated at the end of the trial. The text on page 16 now states this much more clearly as shown below.

“The trial team collects baseline blood samples from all participants during the first visit, after informed consent, to assess levels of serum ionised calcium as well as serum 25(OH) vitamin D assay, HbA1c and the other secondary endpoints mentioned earlier. During the second and third visits blood samples are collected only for safety analysis (as described under the section on safety) to make a judgement about continuation of the IMP. During the final (fourth) visit all blood samples are repeated, as during the first visit. Assays for safety monitoring (serum ionised calcium) at all visits are performed contemporaneously. For other trial endpoint assays, the HbA1c samples from the first and fourth visit are analysed immediately on fresh samples, while aliquots for all other assays (including 25(OH)D) are stored frozen at -70C to be measured at the end of the trial. Thus in summary, blood samples for safety are collected at all four visits, while for other endpoints bloods are collected only at the first (baseline) and fourth (final) visit.”
4- Definition of vitamin D status (deficiency, insufficiency and sufficiency, based on serum cutoffs for 25(OH)D) is needed.

In our statistical analysis plan (http://www.mrc-epid.cam.ac.uk/files/2013/04/VitDtrial_AnalysisPlan_Sep2012.pdf) we have pre-specified that we will examine as secondary outcomes the “proportion of participants with serum 25(OH)D3 in the following categories: <25 nmol/l, 25 to <50 nmol/l, 50 to <75 nmol/l, 75 to <150 nmol/l and >=150 nmol/l. This will enable us to examine the vitamin D status of deficiency (<25), insufficiency (25 to <50 or 25 to < 75 by different definitions) and sufficiency (>50 or >75 by different definitions) as secondary endpoints. We have not listed all the secondary endpoints in the manuscript, but comprehensive information is provided in the statistical analysis plan.

Major issues:

1- I do believe that anthropometric data are necessary. There are some evidence suggesting that vitamin D may affect body weight and even abdominal fat. Needless to say that these two variables can influence many (if not all) of the metabolic variables being tested in this study. It will be quite valuable if estimation of both total and abdominal body fat is also done (at least in a subsample).

Response: We are collecting anthropometric data on all our participants at baseline and at the last trial visit. This includes height, weight, waist:hip ratio, body fat measurement, blood pressure and heart rate. We are not measuring abdominal fat directly, but are using waist:hip ratio as a surrogate measure. We have stated on page 11 (lines 3-4) that we are measuring anthropometric variables as secondary endpoints.

2- Considering the above point, dietary assessment is also necessary.

Response: We are using a standardised questionnaire to assess dietary intake, and we have stated this on page 15 (line 17).

3- You may evaluate the duration of sun exposure via a questionnaire.

Response: Thank you for pointing this out. We are measuring sun exposure using a standardised questionnaire, but had omitted to include this in our description. We have now done so on page 15 (line 17).

4. Level of interest: An article of importance in its field; Quality of written English: Acceptable; Statistical review: Yes, and I have assessed the statistics in my report.

Response: Thank you.

Reviewer 2:

General comments: Vit D is a hot topic, especially its role in diabetes. The present study is therefore of interest. The manuscript is well written. It would be improved if there are more detailed explanation of the issues below.
Response: Thank you. We have addressed the issues below.

1. Please explain why 0.5% was adopted as the standard deviation of A1C. This impacts on the power of the study.

Response: We have stated on manuscript page 17 (lines 8-10) that we used the ProActive trial and the BanglaDip Study to derive the assumptions of the sample size calculations (including the SD of 0.5% for HbA1c), as these represent populations similar to those we are recruiting. We further also used information from the Brent/Southall studies where a similar SD of HbA1c was observed (previously unpublished but now published - Tillin T et al, Diabetes Care 2013, 36:383-393). Please also note that the two laboratories (London site, Cambridge site) that measure HbA1c for the trial adhere to the IFCC standards and report in IFCC units. The final results will be available in both IFCC and DCCT units.

2. For both A1C and blood pressure, lifestyle factors and medications are big confounding factors. Please explain how these are controlled (if at all) and controlled for in the analysis. As a minimum, body weight and medications must be known, whereas dietary vit D, exposure to sunlight and exercise would be hard to measure accurately.

Response: We are measuring both body weight and recording any medication changes during the trial. We are also using standardised questionnaires to assess dietary exposure to vitamin D as well as sunlight exposure. We are using questionnaires to assess physical activity. This information is included on page 16 of the manuscript. The primary pre-specified trial analysis (as per the statistical analysis plan – referenced in the manuscript as available at this address: http://www.mrc-epid.cam.ac.uk/files/2013/04/VitDtrial_AnalysisPlan_Sep2012.pdf) will not be adjusted for any covariates, since any differences between randomised groups at baseline will be due to chance (this approach is consistent with the CONSORT guidelines, www.consort-statement.org).

3. The paragraph on limitations should be expanded.

We have added further limitations as below, in the discussion section (page 22, lines 7-13)

“Finally, this trial is measuring markers of glycaemia (e.g. HbA1c) and of cardiovascular risk (e.g. pulse wave velocity, modelled CVD risk) without ‘hard’ endpoints of incident events of diabetes or cardiovascular disease. While the current endpoints are important in their own right to understand the effects of vitamin D supplementation on these parameters, and indeed we have included adequate sample size to enable us to investigate this appropriately, it will also be important to assess clinical events in specifically designed future trials.”

4. Level of interest: An article whose findings are important to those with closely related research interests; Quality of written English: Acceptable; Statistical review: Yes, and I have assessed the statistics in my report.

Response: Thank you.