Author's response to reviews

Title: Study protocol: A randomized placebo-controlled clinical trial to study the effect of vitamin D supplementation on glycaemic control in type 2 Diabetes Mellitus SUNNY trial

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

Please find enclosed our reply to the points and questions raised by the reviewers of our manuscript. Our reply is organised per reviewer and per point. We have also enclosed a version in which the appropriate changes to the manuscript have been indicated in yellow.

The comments of the reviewers are very worthwhile, however we can’t change major topics of the protocol, due to the fact that the trial has already been approved by the Medical Ethics Committee and just started with the inclusion of participants.

We would highly appreciate your reconsideration for publication of this revised manuscript in BioMed Central – Endocrine Disorders.

Sincerely yours,
Also on behalf of the co-authors,

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Comments to the editor:

During the review process, some concerns have been raised regarding your protocol. Specifically a concern has been raised regarding whether it could be considered ethical to administer the same dosage of vitamin D to patients despite differences in the severity of deficiency. We would be grateful if you could comment on this point in your covering letter.

- We decided to give all patients the same dosage of vitamin D, because it is known that this amount of vitamin D will raise serum vitamin D adequately in case of vitamin D deficiency and in case of vitamin D deficiency this amount will not give any toxicity.

Referee 1:
1. In background section, the author should address the roles of vitamin D in insulin resistance, especially in immunoregulatory function and associated pro-inflammatory cytokine.
   - In line 65 and 66 this point is mentioned. We did not mention this topic more extensively because this association is mostly examined in patients with type 1 diabetes mellitus and is more uncertain in T2DM.

2. The author proposed the diabetic patients in study are excluded when serum 25OHD < 15nmol/l or > 150nmol/l. However, if these patients are supposed to be have sufficient vitamin D, the study design and recruitment flow chart should be revised to study only in patients of type 2 DM with vitamin D deficiency.
   - We chose to include all diabetic patients with a serum level of 25OHD > 15nmol/l and < 150nmol/l. We doubled our calculated sample size with the expectation that 50% of all patients will be vitamin D deficient (< 50nmol/l). Thereby we can do all analyses in vitamin D subgroups to compare the results. This is written in line 125-131.

3. In inclusion/exclusion criteria, other divalent ion disturbances, like hypocalcemia, hyper/hypophosphatemia, abnormality of PTH regulation should be added in exclusion criteria.
   - Thank you for this worthwhile advice, which we added into the protocol (line 111-112).

4. As we know, the oral glucose tolerance test can provide the pancreas beta cell function. We suggest add the test to clarify. Otherwise, the immunoregulatory or pro-inflammatory cytokines are also considered to be addressed in this study.
   - This is a very interesting topic. We could not arrange an oral glucose tolerance test in our hospital for all patients (there was not enough money to do this).

5. The authors design to treat patient with oral vitamin D3 (50,000 IU monthly) for 6 months, however daily oral vitamin D supplement seems to be reasonable.
   - You are right in this point. We chose in our research group for a dose once monthly due to the fact that patients did not want to take an extra pill every day.

6. The authors also detect the skin AGE accumulation by skin autofluorescence during 6 months to evaluate the microvascular or macrovascular complication caused by oxidative stress associated with vitamin D deficiency. However, the study period seems to be so short that AGE may not accumulate during these 6 months. Other biomarkers of oxidative stress, for example serum nucleic acidoxidation of 8-oxo-dG, 8-OH-dG, are suggested to more reasonable.
- This is a very good suggestion and we will try to determine these values in the future as well. It is right that the AGE accumulation won’t change in such a short period. However, the AGE accumulation is of interest at baseline to compare this with other baseline variables as serum 25OHD, lipid spectrum, Hba1c. We changed this accordingly in line 95.

7. The authors should clarify the method and how to presentation of quality of life.
- This is added in line 243-245.

8. This issue had been investigated in previous study. (Ian H. de Boer, Michael Sachs, Andrew N. Hoofnagle, Kristina M. Utzschneider, Steven E. Kahn, Bryan Kestenbaum and Jonathan Himmelfarb. Paricalcitol does not improve glucose metabolism in patients with stage 3–4 chronic kidney disease. Kidney Int. 2013; 83: 323–330) We read this interesting article. However this is another study population and the patients received paricalcitol which is 1,25 dihydroxyvitamin D in stead of cholecalciferol 25(OH)D in our study.

Referee 2:

1. Will the study design adequately test the hypothesis? Compared with most other similar studies now out, many of which are not covered in either the introduction or discussion but should be included one would think so! However, there are some points of information that suggest certain modifications would improve the chances of the study producing answers that will stand up to the test of time, as mentioned in the specific comments listed below.
- We wrote the protocol in 2012 and it was reviewed and approved by the Medical Ethics Committee in 2013. We now updated the literature in the introduction (line 84-87; reference 22-24)

2. Are sufficient details provided to allow replication of the work or comparison with related analyses: if not, what is missing? I believe so; however, there are some aspects of the proposed RCT where additional data inclusion should be considered, as detailed in the specific comments below. Especially important matters to include are:

2.1 Season of study needs more specific information since if the RCT is done winter to summer the change in season, and increased sunlight, may have effects independent of vitamin D status, whilst if done from summer to winter, similar factors might obscure what would otherwise be a positive result.
- We planned to spread the inclusion of all patients over one year to prevent large seasonal influences. This is added in line 214.

2.2 Safety checks are not specifically mentioned. Many RCTs using large interval doses do point of care serum calcium checks early on. The minimum one must offer is the provision of written advice on when to seek advice and about how to get it [contact details etc.] which should be included in this type of RCT and should be stated in the plans to satisfy ethics committees and also to help anyone planning to carry out similar studies, especially where older people and women are included as undiagnosed primary hyperparathyroidism is increasingly common with age and in women more than men
- This is added in line 222 -225.

2.3 There is no mention of an external monitoring committee though this is implied on page 9,
where premature termination of the trial is mentioned. Also, who checks data to see when
individuals develop abnormal responses requiring their withdrawal from the RCT. This
should be stated.
- This will be done by a specialised nurse who is independently related to the research. See
line 222-225.

2.4 How will checks be made that subjects can measure the doses of liquid oral doses
correctly and what will they use for self-dosing? This is a safety matter and should be
specified though it is also important information for others planning any similar RCT
- This is added in line 157-159.

2.5 additional details on how measurements are made of BP, etc should specify the period of
sitting or non-smoking beforehand, and what time weight and height are taken both change
over 24 hr to help anyone planning to emulate this RCT.
- This is added in line 236-238.

2.6 How far the 25(OH)D assays can detect both the D2 and D3 metabolites, i.e. total
25(OH)D, should be stated, together with the CV for these assays and what QC scheme will
be in use. The CVs for all non auto-analyzer data should be stated.
- This is added in line 257-270.

3. Is the planned statistical analysis appropriate? I believe that it is but I am not a statistician
and further opinion is required.
- We have gone through the statistical analysis with a statistician.

4. Is the writing acceptable? On the whole it is but there are remarks that are unclear and need
clarification as listed below in the specific comments
- no comments

5. ALSO, the consort Table should show that numbers in all groups will be given at each
stage.
- We added this in the table.

Specific comments:

Dosing with vitamin D:- monthly has been pretty standard but recent evidence suggests intact
vitamin D3 is 25-hydroxylated in many target tissues increasing availability for activation
locally, thus, with its short half life in the circulation it is likely to be more effective given
daily, matching how early man got his vitamin D from sunlight. Since there is evidence that
daily dosing is being more effective on end points than interval dosing future RCTs may use
this dosing schedule increasingly and maybe this could be done in this RCT.
- This is a good point, however when we designed this protocol there was no evidence yet
which form of dosage is better. We chose in our research group for a dose once monthly due
to the fact that patients did not want to take an extra pill every day.

RE recent literature, RCTs of vitamin D for quality of life in T2DM is novel but there are
several RCTs out in 2013 of vitamin D in T2DM for metabolic benefits, these should be
quoted [in addition to refs 16-21], and since some were effective on T2DM related end points
those end points could usefully be more prominent in the planning
- We updated the literature as mentioned before.
AGEs once formed are irreversible so how is an RCT going to see improvements in the skin – only if skin is replaced significantly within the span of the RCT, so data on this would be helpful AGEs

- You’re right in this point. It is of interest to investigate the AGE accumulation in the skin compared to other baseline characteristics as serum vitamin D, Hba1c, prevalence of cardiovascular disease. We changed this in line 95.

The benefits of vitamin D repletion have mainly been shown in deficiency [<25 nmol/l], so that stratification should include this cut-off. Also it is likely that the earlier that vitamin D deficiency is corrected in the disorders leading to T2DM the more effective it is. Therefore, I am doubtful that any RCT of vitamin D will make large improvements in beta cell function, though IR may be reduced which will be useful. Despite this, a definitive answer on the value of vitamin D in T2DM is needed so that this type of RCT is going to have to be done.

- We agree with this point. Therefore we doubled our calculated sample size with the expectation that 50% of all patients will be vitamin D deficient (< 50 nmol/l). Thereby we can do all analyses in vitamin D subgroups to compare the results. This is written in line 125-131.

Inflammatory changes reduced by corrected D deficiency include reductions to normal in plasma MMP9, first shown in 2002 [Timms et al] and confirmed in several RCTs since then. Since MMP9 is especially important in atherosclerosis, known to be increased in T2DM, it would add value to this RCT if plasma samples could be taken for MMP9 assays.

- This is a good suggestion, hopefully we can measure this in the future when there is money available for additional questions.

Most measures of T2DM and associated metabolic abnormalities vary with season and improvements coincide with when vitamin D status increases, thus, adjusting for season is a real problem since it could obscure changes associated with changes in vitamin D status. It would be useful, therefore, for seasonal changes in all the end points to be examined in the control group to see what adjustments for season may be appropriate in the treated group.

- We planned spread the inclusion of patients over one year to avoid this problem. For the additional metabolic abnormalities we will check the seasonal influences. Thank you for the advices.

3-6/12 is most unlikely to show any changes in AGEs, as above, so mention of earlier evidence on this would be useful in the background.

- As mentioned in the earlier point we changed this in the protocol.

There was ‘no funding’ for this RCT but this RCT must be costing money, where is this coming from?

- The money required for this RCT will be funded from the department of Internal Medicine in the Medical Centre Alkmaar.