Author’s response to reviews

Title: Differential effects of vitamin D2 and D3 supplements on 25-hydroxyvitamin D level are dose, sex, and time dependent: a randomized controlled trial

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Version: 2 Date: 24 Dec 2016

Author’s response to reviews:

Dear Prof Gittoes,

We would like to thank you for considering our manuscript and our reply and to re-thank the reviewers for their time and effort to improve the manuscript. The following is our point-by-point response to the new reviewers’ comments. The changes in the revised manuscript are highlighted.

Reviewer #1:

The authors have chosen to robustly reject a number of recommendations by both reviewers.

Authors’ reply: We don’t find this statement to be true. We benefited from all the recommendations of reviewer #2, who stated in his re-evaluation: “I am satisfied that my original comments have been addressed adequately.” It was not possible for us to accept some of the recommendations of reviewer #1, such as [I think the term is "hypercalcurea."] and [D2 and D3 levels were not determined!]. However, we did accept several of reviewer #1 recommendations and we thanked him (and thank him again) for his intend to improve the manuscript.

While the reviewer is accused of subjectivity in these reflections, recommendations are intended to improve clarity of language and are based on a number of years’ experience as an author, reviewer and editor. They also failed to note the convention that abstracts stand alone and should not use terms defined in the body of the manuscript. Nor should the manuscript depend on terms
defined in the abstract. Hence comments relating to the abstract and the definition of terms therein were made in this context. They appear to have attempted to correct this (mostly).

Authors’ reply: We note that no response is required.

The 1st sentence ("Vitamin D (D) supplements are indispensable for its world-wide deficiency.") is subjective, redundant and poor English.

Authors’ reply: We think that the sentence is objective and important (see for example the Endocrine Society Clinical Practice Guidelines, J Clin Endocrinol Metab, 2011;96(7):1911). It was previously re-phrased based on the reviewer initial comment. We have changed it now to “Supplements of vitamin D (D) are indispensable for its world-wide deficiency.”

Methods: The authors did not randomize anyone! If they insist on using the active voice, then they "randomly allocated treatments to participants". Alternatively, "participants were randomly allocated to treatment with..."

Authors’ reply: If the authors did not randomize anyone, who did?. According to www.merriam-webster.com/dictionary/randomize, randomize means to select, assign, or arrange in a random way. “Randomize” is widely used in the active voice in the medical literature. As examples, Crawford stated “I randomized 109 type 2 diabetics..” (J Am Board Fam Med 2009; 22(5):507), Umpierrez et al stated “We randomized patients with diabetes ... “(Diabetes Care 2015;38(9):1665), Espaulella et al stated “we randomized patients to intervention ..” (Age Ageing 2000; 29(5):425-31), and Riker et al stated “we randomized patients in 5 countries to receive ..” (JAMA 2009; 301(5):489). No changes are made.

The abstract remains confused. The following sentence is confusing. "Controversy continues on ergocalciferol (D2) and cholecalciferol (D3) relative potency as well as on dosing-schedule and sex role in raising 25-hydroxy D (25(OH)D) level, the best indicator of D status." In simple terms of the language used the following may convey the intended sense "There is controversy about the effect of ergocalciferol (D2) and cholecalciferol (D3) on serum 25-hydroxy vitamin D (25(OH)D) levels and how gender and dosing-schedule* may influence this effect." *maybe dosing frequency?

Authors’ reply: We don’t find the suggested sentence a suitable replacement because it changed “relative potency” into “effect” and “sex” into “gender”, and removed “the best indicator of D status”. No changes are made.
In vitamin D research, assay methods are critically important and this should be included in the abstract (as well as with more detail in the main methods section).

Authors’ reply: We have now added “by high performance liquid chromatography assay” to the Abstract (page 2, line 40).

The methods section does not present a clear hypothesis to be tested or how it would be tested. There is a stated primary endpoint (AUC for plasma ?serum 25OHD) which I presume is to be compared between allocated groups.

Authors’ reply: The levels were measured in serum. “Serum” is now added to abstract (page 2, line 39). Thank you. The intended comparison of the primary endpoint is clear from the design and is fully detailed in the results section of the abstract.

In the abstract it should also say whether participants received a daily tablet as a combination of placebo or active ingredient depending on their allocation ( “blinded”) OR they were allocated to receive a daily, fortnightly or monthly dose (clearly not blinded to dose interval). It may be clear to the researchers but not to THIS reader.

Authors’ reply: This sentence in not clear to us. However, we note that the following pieces of information are already in the abstract (page 2, lines 35 to 38). “We randomized 279 adults to daily D2, D3, D2/D3, or placebo; 2-weekly D2 or D3; or 4-weekly D2 or D3 (250,000 IU over/140 days). Randomization sequence, stratified by body-mass-index (BMI) and sex, was concealed from study coordinators and participants who were then blinded to capsules’ content. No changes are made.

The results section remains cluttered. Based on the implied intention to compare AUCs between doses, this should be presented as a result. Were statistical tests performed to compare AUCs?

Authors’ reply: ANCOVA was used as statistical test (please see page 8, line 181). The results of comparing AUCs between different dosing schedules are indeed presented in the result section. Please see page 10, 2nd paragraph for total 25(OH)D; page 12, 2nd paragraph for 25(OH)D2; and page 12, 3rd paragraph and page 13 for 25(OH)D3. [If the reviewer means the results section of the Abstract, please see page 2, lines 42-43 and lines 47-52.] No changes are made.
The authors still say that D2 and D3 were measured. I can not see any evidence that serum or plasma cholecalciferol or ergocalciferol were measured (although of course total 25OHD, 25OHD2 and 25OHD3 were measured and results presented).

Authors’ reply: We have already addressed this obvious point in our previous reply: “Of course they were determined! Please see abstract (page 2, lines 55-59), methods (page 7, line 157), results (pages 14-15), and Figure 6.” We are surprised that the point is raised again. No changes are made.

They insist on using the term "D" rather than vitamin D, citing old references. I see no value in using this jargon term and strongly counsel against it. This is particularly relevant in this study looking at cholecalciferol, ergocalciferol and the metabolites thereof: 25(OH) cholecalciferol, 25(OH) ergocalciferol and total vitamin D (25(OH)D2+25(OH)D3).

Authors’ reply: We have already addressed this point in our previous reply. We note here that the references that we cited were published from 1979 to 2015 (most in the last few years) and that the “age” of the reference should not have negative weight as there is no recent agreement on accepted abbreviations. We are not aware that 25(OH) cholecalciferol and 25(OH) ergocalciferol, which are now suggested by the reviewer, are used in the literature. No changes are made.

I still believe the suggestion that the dose response relationships could be "quadratic or exponential" is far too specific and would demand further discussion and explanation, as these dose responses are not consistent with each other. I would strongly counsel for the use of the term "non-linear" which applies to both.

Authors’ reply: There is no inconsistency, please note that “or” was used not “and” (please also see the comment of reviewer # 3). In fact, the less specific term “non-linear” (not the more specific terms) would demand further discussion and explanation. Nevertheless, we have now changed the sentence to read “Further, the dose-response curve may be curvilinear rather than linear.”(page 4, lines 89-90).

P6L9 The term "partially blinded" remains confusing (see comments above from abstract). as is the response "It means that participants were blinded to the content of the capsules (D2 vs D3 vs D2/D3 vs placebo for daily groups, D2 vs D3 for 2-weekly and 4-weekly groups) but were not blinded to the dose. It is clearly explained under methods (randomisation and blinding, page 7, line 17 and page 8, lines 172-173). No changes are made. In normal English usage, the word "dose" is more generally applied to the a quantity of a medicine given. I would recommend.
"participants were given tablets daily, fortnightly or monthly but were not aware of which supplement or dose they were receiving"

Authors’ reply: In fact, the word “dose” was applied here, just like in normal English, to the quantity of vitamin D given. Participants were blinded to the content of the capsules but not to the dose. For example, participants on the 2-weekly regimen knew (were not blinded) that they are taking 25,000 IU but they were blinded to whether it is D2 or D3.

The recommended sentence is not correct. Please note that participants were given capsules not tablets, that there were no monthly dosing (it was 4-weekly), and that participants on 2-weekly and 4-weekly regimens were aware of the dose they received. No changes are made.

As an RCT some estimate of the power to detect a difference would be expected. This is a weakness of the study design.

Authors’ reply: We have addressed this point in our previous reply and indicated that this was an exploratory study and that this fact was addressed under Limitations section (lines 609-910). No changes are made.

With regard to the definition of the intervention on P7L10 "Daily doses (D2 2000 IU, D3 2000 IU, combined D2 1000 IU and D3 1000 IU, or placebo) on days 0, 1, 2, 3, 4, 7, and 14 and 2-weekly thereafter and all of the 2-weekly (D2 25,000 IU or D3 25,000 IU) and 4-weekly (D2 50,000 IU or D3 50,000 IU) doses": This is the 1st time when the intervention is clarified.

While they respond that this is not correct as the interventions were clarified in the abstract, they miss the point that this should have been placed earlier under "Design" and not "Participants". As for the contents of the abstract: as stated elsewhere, the abstract stands alone.

Authors’ reply: Our previous (complete) reply to this point was “This is not correct. The interventions were clarified in the abstract (page 2, lines 35-36). They were clarified AGAIN here because it is the very appropriate place (methods -procedures and interventions section). No changes were made.”

We would like to emphasize here that the interventions were clarified under “Procedures and Interventions” not under “Participants” as stated by the reviewer and that the interventions were indeed described (briefly) under Design: “Participants were randomly allocated to daily D2, D3, combination of D2 and D3, or placebo; 2-weekly D2 or D3; or 4-weekly D2 or D3. Total D dose in the active treatment groups was 250,000 IU over 140 days.” (Page 6, lines 115-117). No changes are made.
While I have some sympathy with the authors frustration at the challenge presented on the use of HPLC analysis, it is not sufficient to report that this method was used without reference to precision/imprecision of the method, particularly for an "in house" assay. Reference to a historic paper describing the development of the assay (As presented in ref. 38) is not the same as describing its performance. Does the lab reference to external standards? Was the lab a member of DEQAS and/or NIST/NIH Vitamin D Metabolites Quality Assurance Program (VitDQAP)? This is a weakness of the study.

Authors’ reply: We don’t have any frustration with our HPLC assay! We wonder why the reviewer is assuming that. In fact, we are proud of it because it can simultaneously quantitate D2, D3, 25(OH)D2, and 25(OH)D3.

As for the precision/imprecision of the methods, we have indicated in our previous reply that they ARE summarized on page 7. The intra-assay and inter-assay CVs are clearly stated for D2, D3, 25(OH)D2, and 25(OH)D3 along with the lower detection limits and lower quantitation limits (lines 158-164).

We don’t know what the reviewer implies by “Reference to a historic paper describing the development of the assay (As presented in ref. 38) is not the same as describing its performance.” First, the assay is not historic; it was published in 2012. Second, the paper does not only describe the development of the assay but also its PERFORMANCE and full VALIDATION. The assay was developed and validated in our lab for the purpose of this study. Our lab is not a clinical lab and has not participated with DEQAS or VitDQAP. We think that this has little bearing on our findings and conclusions, as all samples were analyzed using the same assay. We also note that the changes we observed in 25(OH)D level are consistent with the literature (Please see lines 423-433). No changes are made.

Clearly the author rejects a number of other recommendations made which were intended to improve the clarity of the text and present the concepts behind the study and the results. I would strongly recommend the authors look at the work of Professor Sue Lanham-New, who has recently performed controlled trial of vitamin D2/D3 supplementation.

Authors’ reply: Thank you. We note that no specific comments were made.

Reviewer #2:

I am satisfied that my original comments have been addressed adequately.

Thank you.

Authors’ reply: Thank you.
Reviewer 3: General comment:

This study compares the vitamin D dose response in terms of 25OHD concentrations to both vitamin D2 and vitamin D3 giving daily, 2-weekly, or 4-weekly. The study design, performance, and analysis are conducted to the highest standard as is the discussion and review of the relevant literature.

Authors’ reply: Thank you.

The issue of generalizability (Lines 607-609) needs to be expanded. A major limitation of the work that needs to be understood first and then expressed in the manuscript is the generalizability of their findings in the context of vitamin D intake requirements as recently deliberated in North America for the USA and Canada by the Institute of Medicine (IOM) in the 2011 report on "Dietary Reference Intakes for Calcium and Vitamin D" and in the UK by the Scientific Advisory Committee on Nutrition (SACN) in 2016 report "Vitamin D and Health" (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/537616/SACN_Vitamin_D_and_Health_report.pdf). The estimated average requirement (EAR) according to the IOM is 10 µg/day (400 IU/day) and the reference nutrient intake (RNI) according to SACN is also 10 µg/day (400 IU/day). Furthermore, according to IOM the EAR specification is for those with minimal sunlight exposure (such as housebound individuals), and the specification refers to total oral vitamin D intake not just supplemental intake. SACN makes a similar statement about the uncertain effect of sunlight exposure on determining the oral intake requirement. The supplemental dose in this study is 4.5-fold higher than the EAR and the RNI. Secondly, vitamin D status in the study population with average 25OHD at about 40 nmol/L already has an estimated total vitamin D intake (from oral and sunlight sources) equivalent to the EAR, because the IOM indicated that the corresponding 25OHD for EAR is 40 nmol/L (1-3). Thus, the findings of this study are not generalizable to populations at risk of vitamin D deficiency for whom public health measures regarding fortification and supplementation are needed to address the problem. The IOM specifications have been consistently misrepresented in the medical literature (4).

Authors’ reply: We are fully aware of the IOM 2011 report and the controversy about it. We note that the IOM report is on dietary reference intakes of vitamin D (and calcium). However, our study was not designed to address the recommended estimated average requirement (EAR, corresponding to the median intake needs of the US and Canadian population), recommended dietary allowances (RDA, corresponding to 2 SD above the median needs that would cover the needs of 97.5% of the same population), or upper intake level (UL, the highest intake that is likely to pose no risk). Our study simply compared the relative potency of several vitamin D regimens in raising 25(OH)D level.
We also note that the IOM RDA (not EAR) is 600 IU/d for adults less than 70 years old and 800 for older adults (not 400 IU/d), while the UL is 4000 IU/d. Such RDA corresponds to 25(OH)D level of at least 50 nmol/l even with minimal sun exposure as per IOM.

Although both the IOM and the Endocrine Society Clinical Practice Guidelines (J Clin Endocrinol Metab 2011;96(7):1911) define vitamin D deficiency as a 25(OH)D below 50 nmol/l, the Endocrine Society has an additional category of vitamin D insufficiency (25(OH)D level less than 75 nmol/L). Further, the Endocrine Society recommends 1500-2000 IU/d to raise the blood level of 25(OH)D consistently above 75 nmol/L.

The mean 25(OH)D level in our participants was about 40 nmol/l which is clearly in the vitamin deficiency range. The dose in our study was 1786 IU/d (for 140 days, not life-long), which is 2.2 to 3 fold the RDA required LONG TERM to maintain 25(OH)D level above the deficiency limit, exactly what appears to be required to maintain 25(OH)D level above the insufficiency limit (J Clin Endocrinol Metab 2011;96(7):1911), and less than half the UL.

We have now modified the following sentence under Limitations: “Another limitation of the study is that our findings may not be generalizable to subjects with different baseline 25(OH)D levels, with different demographics, or with co-morbidities.” to “Another limitation of the study is that our findings may not be generalizable to lower or higher doses of vitamin D, or to subjects with different baseline 25(OH)D levels, with different demographics, or with co-morbidities.” (page 23, lines 614-617).

Another limitation is the difference in adherence between the 3 groups. Given that the findings of the study are counterintuitive in that the daily dosing is less potent than both the 2-weekly and sometimes the 4-weekly, suggests problems with adherence. The 2-weekly and 4-weekly doses were administered under supervision, but the daily doses were self-administered with adherence being dependent on capsule counting. Although compliance was recorded as being high, there is uncertainty about reliability, which needs to be stated. Given that 2-weekly dose response was superior to 4-weekly dose response, this would support the frequent finding in the medical literature that higher doses at less frequent intervals have a lower dose response compared to equivalent but lower doses given at shorter intervals. If administration of daily doses were supervised, then the dose-response would be at least equal to if not superior to both 2-weekly and 4-weekly dosing.

Authors’ reply: The issue of compliance with daily regimens was addressed under Limitations. We agree that capsule counts may not be an accurate assessment of compliance. However, we would like to point out that daily doses were inferior to 2-weekly and 4-weekly doses only for D3 regimens. For D2 regimens, the daily doses were superior to both 2-weekly and 4-weekly doses (please see Figure 2, Figure 4, And abstract, page 2, lines 49-52). Further, participants on daily regimens were blinded as to the content of the capsule (placebo, D2, D3, or D2/D3), thus it
would be difficult to attribute the findings to incompliance (why should participants randomized to D2 be compliant but participants randomized to D3 not compliant?).

We have now added the following sentence to clarify this important point: “Although the incompliance rate was low, it was measured by capsule count, which may not be reliable. Thus the lower dose-response observed with daily D3 treatment compared to 2-weekly and 4-weekly D3 treatments could be explained at least in part by incompliance. However, such explanation is not likely given our observation that the dose-response was higher with daily D2 treatment compared to 2-weekly and 4-weekly D2 treatments and the fact that assignment to daily D2 or D3 treatment was random and blinded. (page 22, lines 601-608).

Specific Comment

In lines 88-89 the authors quite correctly state that "the dose-response curve may follow a quadratic or exponential rather than linear function." Yet, in lines 422-431, the authors interpret the dose-response as a rate constant. While correct when making comparisons of dose-response between populations given similar doses and having identical 25OHD, it is a flawed approach. The IOM using a simulated model demonstrated that the dose-response was defined by a logarithmic function (1). The ViDOS study using different doses in an RCT showed that the dose-response was best defined by a quadratic function (5). Interpreting the dose-response as a linear function rather than as a curvilinear function leads to gross overestimates of intake requirements (6). In simple terms, the clinician needs to know the total intake dose (of either D2 or D3) that is required to achieve a certain 25OHD rather than endorsing a fixed linear dose-response; this is one of the paradigmatic shifts in understanding vitamin D intake requirements that arose from the IOM report. By contrast to this section, the authors in lines 491-506 address comprehensively the many factors that account for individual variability in the dose response.

Authors ’reply: The discussion on lines 423-432 (previous version) is meant to show consistency of our findings with the findings of previous studies. We did indicate there the doses used and baseline 25(OH)D levels in different studies. To emphasize the important point raised by the reviewer, we have now added the following “Although comparison is difficult because the dose-response curve is curvilinear;” (lines 425-426).

Lines 88-89: It should be changed to "Further, the dose-response is curvilinear not linear (1, 5)."

Authors’ reply: The sentence is now changed to “Further, the dose-response curve may be curvilinear rather than linear.”(Page 4, lines 89-90).
I hope that the revision is acceptable and would like to thank you again for your consideration.

Best regards and happy holidays,

Muhammad M Hammami, MD, PhD