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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Author’s response to reviews:

Dear Prof Gittoes,

We would like to thank you for considering our manuscript for publication and to thank the reviewers for their time and effort to improve the manuscript. The following is our point-by-point response to the reviewers’ comments. The changes in the revised manuscript are highlighted. We would like to point out that some of the comments of reviewer#1 are rather subjective and more related to personal preferences than to critical appraisal. Nevertheless, we have tried to accommodate them as much as we could.

Reviewer #1: Abstract:

The abstract is confusing with a scattering of jargon terms and a lack of clarity.

Specific comments:

1. Background

"D deficiency" is not an acceptable term. Here (and throughout) "vitamin D deficiency" should be used.

AR: We used “D” as an abbreviation of vitamin D. This abbreviation was defined in the abstract (page 2, line 30), the background (page 4, line 76), and under list of abbreviations (page 24, line 642). Further, “D” as an abbreviation of vitamin D and was used in the literature at least since 1979 (Please see Whyte etal, JCEM 1979; 48(6):906-11). Nevertheless, we have changed the sentence to read “Vitamin D (D) supplements are indispensable for its world-wide deficiency”.


2. The abbreviations D2 and D3 need to be defined

AR: They were defined under background (page 4, lines 78 and 79) and under list of abbreviations (page 24, lines 643-644). We have now added their definition to the abstract. Thank you.

3. English is poor here: suggest, for example, "the relative potency of vitamin D2 and vitamin D3 and the appropriate dosing schedule for supplementation to improve serum 25—hydroxyl vitamin D..."

AR: We have to disagree. “appropriate dosing schedule” is neither clear nor correct. Further, there is no mention of the sex role in the proposed sentence. However, we have now added “as well as on” to simplify the sentence.

Methods:
4. It is convention to use the passive voice rather than "We randomized…"

Whose convention? In fact, passive voice is in general discouraged. No changes were made.

5. D2 and D3 levels were not determined!

AR: 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.

6. Primary endpoint was the AUC of what?


7. Adjusted for sex, BMI and baseline level of what?

AR: Baseline of 25(OH)D. “25(OH)D” is now added (abstract, page 2, line 42). Thank you.

8. How was 25(OH)D measured?
AR: By HPLC, please see page 7, lines 157-165.

Results:

9. When referring to baseline 25(OH)D is this the total serum 25(OH)D, comprising serum 25(OH)D2 and serum 25(OH)D3?

AR: Yes. It was listed under abbreviations (page 24, line 645). It is now also defined in the abstract (page 2, line 41). Thank you.

10. Some definition of terms "AUC140", "AUC7" etc. are required as they appear to be typos!

AR: They are not typos. They were fully defined on page 8 (lines 176-180) and listed under list of abbreviations (page 23, lines 634-637). They are now also defined in the abstract (page 2, lines 46-47 and line 55). Thank you.

P4L7 I do not think that "D" is an appropriate abbreviation for "vitamin D". Here (and throughout) the term "vitamin D" should be used.

AR: Why not? A quick review of the literature shows that “D” has been used as an abbreviation for vitamin D (please see above), that “D2” and “D3” have been used as abbreviations for vitamin D2 and vitamin D3 (see for example references 4,9,27,41,42), and that “25(OH)D” has been used as an abbreviation for 25 hydroxyvitamin D (see for example references 1,6,7,9,18,27,33). As indicated above all these were abbreviations were defined in the text and listed under the list of abbreviations. No changes were made.

P4L11 "D-type" is an ugly term! Moreover, there is no suggestion that vitamin D2 is preferable. The question is about bio-equivalence!

AR: The question is not about bioequivalence! Bioequivalence refers to comparing the rate and extent of absorption of two formulations of the same dose of the same drug. Here we have two different drugs and several different doses and we are not examining absorption only.

We have now changed “the preferable D-type” to “the relative potency” (background, page 4, line 78). Thank you.
P4L17 "Current unitage… " should read "The same units are used for both vitamin D2 and D3, suggesting biological equivalence"

AR: The sentence is now changed to read “The same unit is used for both D2 and D3, suggesting biological equivalence in terms of raising serum 25-hydroxy D…” (page 4, line 81). Thank you.

P4L32 For "may follow a quadratic or exponential rather than linear function" I suggest "may be non-linear"

AR: We prefer to keep the sentence as is because it provides more specific information. No changes were made.

P4L35 D2 and D3 should read "Vitamin D2 and vitamin D3"

AR: Please see our reply above to the comments P4L7. No changes were made.

P4L39 A number of contentious assertions are made here e.g. differences in plasma (or serum) half-life, for which there is scant evidence.

AR: We did not make any contentious assertion. These are referenced statements (please references 24 and 28). No changes were made.

P4L40 "1,25(OH2)D2" should be "1,25(OH)2D2" and "1,25(OH2)D3" should be "1,25(OH)2D3"

AR: Done all through the manuscript, thank you.

P4L49 instead of "increases" I suggest "increments"

AR: Done, thank you.
The suggestion that circulating colecalciferol or circulating ergocalciferol (which is how I read this sentence) having an important physiological role in many tissues is extremely contentious and, I believe, beyond the scope of this study!

AR: This statement is based on published data (Hollis et al, our reference 3). It is very relevant to our manuscript since we are comparing the two types of vitamin D and different dosing regimens. Please see page 21, line 559-572 under discussion. No changes were made.

for "25(OH)D levels" I wonder whether the term "Total 25(OH)D levels" would be preferred, reflecting the sum of 25(OH)D2 levels and 25(OH)D3 levels?

AR: As we pointed out above, 25(OH)D level is commonly used to indicate total 25(OH)D levels (please see references 7 and 27 as examples). This is now further clarified under abstract (page 2, line 41), background (page 4, line 82) and list of abbreviations (page 24, line 645).

"The primary aim of this study was to systematically evaluate the relative efficacy of various dosing strategies of D2 and D3 oral supplements in raising 25(OH)D levels." This is not a primary objective. We need a simple null hypothesis. "The primary aim of this study was to evaluate the dose response of total 25(OH)D levels to D2 and D3 oral supplements.

AR: It is not clear to us why it is not primary objective!

We think that OUR primary aim reflects our study design, conduct, and analysis much better than the REVIEWER’s “simple null hypothesis”. The proposed sentence does not indicate that different dosing regimens were compared. [By the way, this is not how hypotheses are stated; and the proposed statement is related to a scientific hypothesis rather than to a null hypothesis.] No changes were made.

The term "partially blinded" is used here. What does it mean?

AR: 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 (randomization and blinding, page 7, line 17 and page 8, lines 172-173). No changes are made.

There does not appear to have been an attempt at a power calculation or an estimation of what represents a clinically or statistically significant difference.
AR: This is correct. The study was exploratory. However, the exact p values were reported.

P6L46 strange to define D2 and D3 at this stage!

AR: Please note that this was not the first time D2 and D3 are defined in the manuscript. This was a re-definition. Nevertheless, we now removed “(D2) and “(D3)” (methods, page 6, line 134). Thank you.

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.

AR: 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.

P7L15 I do not understand why "blood samples were obtained and a standardized meal was given."

AR: This is the methods section (procedures and interventions). Blood samples were obtained in order to measure D2, D3, 25(OH)D2, and 25(OH)D3 levels. A standardized meal was given because meal content affects the absorption of vitamin D (see reference 25). No changes were made.

P7L26 "out-of-the-ordinary sun exposure" is not well defined!

AR: It means out-of-the-ordinary for the individual participants. We have changed it now to “more than habitual” (page 7, lines 152-153; page 9, line 204). Thank you.

P7L34 HPLC is no longer an appropriate way to measure or discern between vitamin 25(OH)D2 and 25(OH)D3, never mind measurement of serum vitamin D2 or serum vitamin D3. This approach needs validation and more QC data!
AR: This is a contentious assertion! The assay used clearly separates 25(OH)D2, 25(OH)D3, D2, and D3. The paper describing the assay is published and is referenced (please see reference 38). The validation results are summarized here (page 7, lines 157-165). No changes were made.

P8L12 term "AUC140" is defined for the first time, I believe (although it has already been used!)
AR: This is methods section (outcome measures and analysis); it is the appropriate place to define the outcomes (page 8, lines 176-179). It is now also defined in the abstract (page 2, lines 46-47). No changes were made.

P8L14 I think the term is "hypercalcurea."
AR: No, the correct term is hypercalciuria.

P8L15 "AUC from day 0 to 7, day 0 to 14 and day 0 to 28 (AUC7, AUC14, and AUC28) were also calculated. AUCs were analyzed using analysis of covariance" does not define the term(s) for which AUC is estimated!
AR: The terms are indicated just 2 lines above this sentence (page 8, lines 177-178). They are 25(OH)D2, 25(OH)D3, D2, and D3. No changes are made.

P9L7 "Two hundred seventy nine participants were randomized to 8 groups" is not justified in terms of the ability to detect a difference between SO MANY groups!
AR: This was the design of the study. It was necessary in order to be able to perform the planned comparisons (D2 vs D3, each with three different dosing regimens vs D2/D3 vs placebo). The limitation imposed by sample size was clearly discussed under “limitation” (page 22, line 593). No changes were made.

Results section rambles on without a clear message, which is not surprising, since there is not a clear hypothesis!
AR: We disagree. We think that the results section is well organized with three subheadings with clear messages: “Differential effects of vitamin D regimens on 25(OH)D level”, “Differential effects of vitamin D regimens on 25(OH)D2 and 25(OH)D3 levels”, and “Differential effects of vitamin D regimens on D2 and D3 levels”. No changes were made.
What does "partially-blinded" mean?

AR: This a repeat question that was addressed in our reply to the comment p6L9. Please see above.

Reviewer #2: Review Comments BMC Endocrine Disorders

This paper is robust in its approach to the question of the effect of different vitamin D supplementation regimens on circulating vitamin D levels. It uses a blinded placebo-controlled randomized control trial design, with end-points as registered with ClinicalTrial.org. Multiple dosing regimens arms are compared, including daily, 2 weekly, 4-weekly D2 or D3. Supplements were subjected to in-house analysis. Assessments at levels of compliance were also made. The study is also informative due to the geographical location of the investigating centre. The majority of vitamin D supplementation studies have been carried out in Europe and the US. The manuscript is on the whole well written and clear.

AR: THANK YOU! We note that no changes are required.

The findings with regards to decreases in D2/D3 levels when treated with the alternative form of vitamin D as shown in Figure 5 are interesting. As a reader it would be interesting to include some speculation on putative mechanisms of this observation.

AR: Thank you. The putative mechanisms are described under discussion (page 18, lines 469-489) and under conclusions (page 23, lines 619-625).

The headline observation 2-weekly and 4-weekly D3 supplementation are superior in increasing serum vitamin D levels as compared to alternative regimens provides further information in this area.

AR: Thank you. We note that no changes are required.

Study limitations include:

Findings may not be generalizable to every patient group setting (in particular outcomes could vary according to baseline vitamin D status, latitude, age, co-morbidities etc), a comment to this effect would be useful.
AR: Thank you. A statement to this effect is now added to the limitation section (page 22, lines 607-608).

In view of the density of data, a potential limitation is in multiple comparisons being made, so the results could be caveated in this way. In particular with regards to secondary end-points of AUC measures at earlier time-points.

AR: Thank you. A statement to this effect is now added to the limitation section (page 22, lines 609-610).

The study design did not allow for full assessment of important adverse effects (such as falls risk in the elderly), so superiority/ inferiority of dosing strategies are restricted to commenting on effect on serum levels only and not on general health effects.

AR: Thank you. A statement to this effect is now added to the limitation section (page 22, lines 611-612).

Many of the study findings replicate those seen previously.

AR: That is true and provides assurance. However, as indicated above by the reviewer, we have also several novel findings, including that the effects of D2 and D3 supplements on 25(OH)D level may be dosing-schedule, time, and sex dependent; that D2-associated reduction in 25(OH)D3 level may be related to the increase in 25(OH)D level rather than being D2-specific; that D2 may be 25-hydroxylated faster than D3; that the association between BMI and response to D supplement may be more pronounced with D2 and during the first few weeks of treatment, and that D2 and D3 level in response to treatment are higher in females compared to males.

Action points for authors:

1. Comments on the above raised study limitations

AR: Done, please see above.

2. A comment on the ethnicity of participants would be informative.

AR: A statement in this regards is now added under results (page 9, line 199). Thank you.
3. There is a discrepancy in the wording of the number of study participants randomized (n=269 vs 279) between the abstract and the first line of results in the manuscript, which is explained by the drop-out rate could this be clarified?

AR: Thank you. Yes, this was because of the drop out of 10 participants. To avoid confusion, we have now added the following statement to the abstract (page 2, line 40): “The results of 269 participants were available for analysis” and changed “We randomized 269..” to “We randomized 279” (page 2, line 35).

Editorial Policies

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Declarations

- Ethics approval and consent to participate
- Consent to publish
- Availability of data and materials
- Competing interests
- Funding
- Authors' Contributions
- Acknowledgements
- Authors' Information

AR: A declaration section (with all of the above sub-sections) was included (pages 23-25).

I hope that the revision is acceptable and would like to thank you again for your consideration.

Best regards,

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