Reviewer’s report

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

Version: 1 Date: 29 Oct 2016

Reviewer: Terry Aspray

Reviewer's report:

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

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

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..."

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?

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).

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. 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.
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?

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).

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).

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.

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"

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

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.

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.

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.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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Not suitable for publication unless extensively edited

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