Reviewer’s report

Title: Vitamin-D2 treatment-associated decrease in 25(OH)D3 level is a reciprocal phenomenon: a randomized controlled trial

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Reviewer: Jonathan Tang

Reviewer's report:

This is an interesting study described by Hammami et al. The hypothesis was intended to assess whether high dose vitamin D2 treatment attenuates the production of 25OHD3, and vice versa with D3 treatment on 25OHD2. The study is appropriately designed and executed, but somewhat underpowered due to the number of arms involved. Outcome measures were based on serum measurements of 25OHD3 and 25OHD2, no physiological response to the vitamin D supplementation was determined.

The study was divided into two arms, study arm 1 was randomised into subjects given either 50,000IU D2 or placebo; in study arm 2 all subjects were treated with 50,000IU D2 before randomisation into 50,000IU D3 or placebo. The authors go on to show that there were indeed reduction in serum 25OHD3 in study 1 and serum 25OHD2 in study 2, from 28 days after vitamin D treatment. The findings are interesting, and it adds sufficiently new scientific knowledge about the pharmacology of vitamin D supplementation. There are a number of issues to be addressed:

General comments:
In study 1, 25OHD3 levels dropped after D2 treatment is likely to be attributed to the D2 loading. However, 25OHD3 did not return back to baseline levels, in fact, the reduction appears to be persistent (up to 56days). If the reduction of 25OHD is a treatment effect, one would expect the levels to return to baseline. It looks like there is a secondary process in place preventing the recovery of 25OHD3. The phenomenon is highly interesting and warrants further investigation and discussion. Measuring 24,25-dihydroxyvitamin D3/D2 in all the time points will help determine the precise mechanism.

For study 2, the conclusion on the D3-associated decline in D2 requires further evidence, as there are several confounding factors at play. Firstly there is no evidence of reversal of 25OHD2 observed similar to that as described above. Secondly, as the subjects in the treatment arm had been given such high doses (50,000IU D2 followed by 50,000IU D3 within 4 days) The observed effect could be a result of total vitamin D overload resulting in rapid elimination to 24,25-dihydroxyvitamin D3 (via 24-hydroxylase stimulated by CYP24A1). To suggest the reduction of 25OHD3 is a D3 treatment effect only maybe overreaching.

Specific comments:
Participants section:

* Participant lifestyle habits - no indication on how the data was collected (?questionnaire), and recruitment criteria.
* Screening 25(OH)D3 (and total 25(OH)D) level >40 and ≤65 nmol/L (study-1) or total 25(OH)D ≥20 and <40 nmol/L (study-1). ?both refer to study 1 - typing error?

Procedures and Interventions

* State purity of the vitamin D capsules and the manufacturing sources of origin (animals/plants).
* HPLC assay performance - state concentrations at which the intra- and interassay CVs were determined. Though method paper is referenced (2012 paper), a brief summary on the HPLC detection method and sample extraction would be useful.
* As mass spectrometry is not the method of choice here, state assay selectivity, and (if any) cross assay interference between D3 and D2 metabolites.

Table 1

* 25OHD2 levels in study 1 should read <12.5 nmol/L, reflecting the LoQ level. To state zero is misleading. Same goes with points in figure 5a.

Figure 2a-d

* Error bars needed to show either SEM or upper and lower percentiles.

Results

* Describing changes in numeral values must state mean (SD) followed by statistical significance. E.g. results section para 4 - "little change" - lacks evidence.
* 7th para, mentioned correlation graphs for day-56 changes - data not shown?
* Rho values on figure 5 - figure legend indicated negative rho values but text says otherwise? Typo?

Figure 5

* Correlations not demonstrated on graphs. Add trend lines, 95% confidence interval of estimate, insert mean line on y axis to the scatterplots.
* Placebo and intervention datapoints are distinguishable on the graph, could this be the reason for the weak correlation? Suggest performing statistical analysis of the placebo and intervention groups separately.
* Fig 5a rho value could be influenced by the placebo groups (see comments above). 25OHD2 values under the assay detection limit should be excluded from statistical analysis, rather than being used as zero.

Discussion

* Para 1, line 1-9 - repeating a summary of the aims and methods are unnecessary. Begin paragraph on major findings.
* Para 5, authors observed a difference in the slopes of decrease in 25OHD2 between study 1 and 2, and commented on the rate of decline is sharper in study 1 than study 2. This is not presented clearly in the graphs and requires further clarification.

Minor corrections (use of language)

* Use until instead of 'til' throughout.
* Use of word 'about' before a value showed uncertainty. Should be expressed as value followed by SD/SEM and statistical significance
* Spelling mistake - 'associated' in para 6 in discussion

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

Yes

**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.

Yes

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