Author's response to reviews

Title: Pharmacokinetics of a single oral dose of vitamin D3 (70,000 IU) in pregnant and non-pregnant women.

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Author's response to reviews: see over
August 14, 2012

Re: “Pharmacokinetics of a single oral dose of vitamin D3 (70,000 IU) in pregnant and non-pregnant women” (MS: 1999557305707617)

To The Nutrition Journal Editorial Team,

Thank you for the opportunity to revise and resubmit our manuscript entitled, “Pharmacokinetics of a single oral dose of vitamin D3 (70,000 IU) in pregnant and non-pregnant women” (MS: 1999557305707617).

As requested, we have responded to the reviewers’ comments in a point-by-point format below, and are submitting for your review a revised manuscript that incorporates the points below and contains a Discussion section shortened in length by ~30%.

We look forward to your reply.

Sincerely, on behalf of the authors,

Daniel Roth
Review Comments and Responses

Reviewer #1: Adekunle Dawodu

1. The authors provided data and discussed safety issues related to a single dose vitamin D3 supplementation. However, since the main aim of the study is to inform antenatal vitamin D3 supplementation regimen in future trials, the authors need to discuss how the results will help in defining dosing regimens of future antenatal vitamin D3 supplementation trials.

RESPONSE: This study provided data that suggest that doses of up to 70,000 IU could be included in prenatal vitamin D3 regimens in the study population. Furthermore, the single-dose pharmacokinetic profile provided assurances that the average pharmacokinetic response during pregnancy conforms to expectations based observations of non-pregnant adults. As such, we can conclude that data from non-pregnant adults can be cautiously used to inform antenatal trial design. These implications are summarized on page 19 of the revised manuscript.

2. It is important to clarify why the dose of 70,000 IU of vitamin D3 was chosen and describe how the sample size was calculated.

RESPONSE: A dose of 70,000 IU was selected to be intermediate between the doses previously studied in the only two rigorous single-dose vitamin D3 pharmacokinetic studies involving non-pregnant adults published at the time of study design (50,000\(^5\) and 100,000 IU\(^\)\(^6\)), thus providing reassurance in terms of probable safety as well as enabling coherent between-study comparisons. Based on previously published pharmacokinetic data in non-pregnant adults, we estimated that 70,000 IU would lead to a statistically significant mean peak rise in \([25(OH)D]\) of at least 25 nmol/L with a sample size of ~15 participants in each group.

We chose to omit this rationale in the interest of text space considerations, but will add it to the text if considered necessary by editors/reviewers.

3. Abstracts: Paragraph 3. The authors noted differences between pregnant and non-pregnant women in the rate of rise of 25(OH)D on days 2 and 21 and provided data on the overall 25(OH)D increase above the baseline in the first month. However, it was not clear whether these results were based on the 61 subjects in the study.

RESPONSE: Sample sizes for each of the analyses are shown in Table 1. For example, differences on days 2 and 21 included data from 27 and 14 women, respectively.

4. Did the authors examine the effect of total body fat on pharmacokinetics of vitamin D3?
**RESPONSE:** No, this was not an aim of the study, and we did not have measures of body fat in the participants.

**Reviewer #2: Paul Lips**

1. Interesting study, well performed, but too long. The number of tables and the text can be reduced to half, and the deleted tables can be made available through the website.

**RESPONSE:** Based on the recommendation of the Editors, we have substantially reduced the length of the Discussion.

**Reviewer #3: Robert Heaney**

**Major Concerns:**

1. The reviewer understands that there were actually two studies going on, one of a single dose followed for approximately 10 weeks, and the other of repeated weekly doses. Only data accumulated during the first seven days of the weekly dose study were used for this paper. However, since both Cmax and Tmax do not occur within the first seven days after dosing, it is not clear how the data accumulated in the first seven days in individuals from the weekly dose study can be validly or usefully combined with the single dose data. Nor is it clear that the paper would suffer if their data were omitted entirely. If the first 7-day data are to be retained, the fact of the second study with a different regimen is irrelevant, and it would be sufficient to say simply that 10 week data were obtained on 31 individuals, and one week data on an additional 30 (or whatever the precise numbers may be).

**RESPONSE:** The reviewer’s understanding of the design is correct, i.e., that only data from the first 7 days of follow-up of the weekly-dose group were used in this analysis. Although these participants did not contribute to estimates of individual Cmax and Tmax (bottom half of Table 1), they were used in the group-level modeling exercises, as presented in Table 3 and Figure 3, and are thus retained in the revised manuscript. The reason to make it clear that there were two separate cohorts was that there were important distinctions between the women with 10-week data and those with 1-week data (e.g., season of enrolment). Also, we wanted to explain, albeit briefly, that a staged approach was taken (i.e., non-pregnant participants prior to pregnant participants; single-dose trials before weekly-dose trials) to ensure the safety of participants. As well, full data from the weekly-dose study will be presented elsewhere, so readers should be aware of the overlap between the two analyses.

2. The second concern relates to the length of the paper. Some way needs to be found to shorten both the Methods (8 pages!) and the Results and Discussion. Removing the description of the second study would be one small part of the shortening.
**RESPONSE:** Based on the recommendation of the Editors, we have substantially reduced the length of the Discussion. Because many readers within the field of nutritional sciences may not be familiar with pharmacokinetic analyses, we felt that the detailed explanation of the methods remained essential.

**Minor Concerns:**

3. Abstract – The Abstract needs explicit quantification of the baseline 25(OH)D value in both groups.

**RESPONSE:** This information has been added to the Abstract.

4. Page 8, line 4 up from bottom – “14” is a Reference citation, and needs parentheses.

**RESPONSE:** Corrected as suggested.

5. Page 5, second paragraph, line 3 – Change the word order to: “. . . only those participants . . .”

**RESPONSE:** We have edited this sentence to improve clarity.

6. Table 2 is probably unnecessary, as there were no significant differences between the groups. (Yes, the entrance 25(OH)D values were different, but there is no point in computing the probability for them, inasmuch as study design was responsible for the difference, not random chance.)

**RESPONSE:** We appreciate this point, but considered that readers would wish to understand the characteristics of the study participants. However, we defer to the Editors for a recommendation as to whether this table should be retained.

7. Table 3, the entry for half-time – How can the number be 46 days overall, while the two components of the total are 44 and 44, respectively?

**RESPONSE:** Three separate models were constructed for all participants, pregnant participants, and non-pregnant participants. So, the estimates for all participants were not necessarily an average of the sub-groups. As well, differences may in part be due to rounding.

8. Figure 2 could be omitted.

**RESPONSE:** We have found that other readers have benefited from this schematic, so retained it.
9. Page 13, line 10 – Do the authors mean milligrams or micrograms? The same question arises on page 17, lines 10 and 12.

RESPONSE: milligrams.

10. What is the justification for computing the geometric mean for serum 25(OH)D? The geometric mean is particularly appropriate for a group of ratios or fractions, such as Ca:Cr, but there is no such ratio for 25(OH)D concentrations.

RESPONSE: The rationale was that the 25(OH)D distribution was skewed (page 9).

11. Page 17, top paragraph – The explanation given here for toxicity is incomplete and probably incorrect. The best explanation for acute toxicity is the fact that a large dose of cholecalciferol, while still in the circulation, saturates the DBP and displaces calcitriol, thereby increasing its free concentration (which, because of bypassing of physiological controls, produces classic toxicity). For toxicity produced by steady state dosing, it is correct to say that the toxicity coincides with the peak 25(OH)D values. But that may not be true (and probably is not) for acute toxicity, in response to very large single doses. As the authors’ own data show, 25-hydroxylation in many individuals can be quite slow, and in the early days after a very large cholecalciferol dose, most of the binding sites on DBP will be occupied by cholecalciferol, not 25(OH)D. That kind of toxicity can be suspected only from direct measurement of the high serum level of the cholecalciferol and/or by measurement of free calcitriol.

RESPONSE: We appreciate this comment, and recognize the uncertainty around mechanisms of vitamin D toxicity. In line with the overall recommendation to reduce the amount of text, we have omitted the statements to which the reviewer’s comment applies.

Reviewer #4: Samantha M Kimball

1. What about the relevance of the dose?

RESPONSE: Same response as for reviewer #1 - A dose of 70,000 IU was selected to be intermediate between the doses previously studied in the only two rigorous single-dose vitamin D3 pharmacokinetic studies involving non-pregnant adults published at the time of study design (50,000⁵ and 100,000 IU⁶), thus providing reassurance in terms of probable safety as well as enabling coherent between-study comparisons. Based on previously published pharmacokinetic data in non-pregnant adults, we estimated that 70,000 IU would lead to a statistically significant mean peak rise in [25(OH)D] of at least 25 nmol/L with a sample size of ~15 participants in each group.

2. Why was 70 000 IU chosen?
**RESPONSE:** See above.

3. What is the clinical benefit? i.e. Is the difference between baseline and end-of-study 25(OH)D concentrations clinically significant? Is the vitamin D status of these women, pregnant or not, changed with respect to "sufficiency" or "deficiency?"

**RESPONSE:** An assessment of clinical benefit was not the aim of this pharmacokinetic study.