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
November 7, 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).

We have responded to the reviewers’ helpful comments in a point-by-point format below, and are submitting for your review a revised manuscript.

We look forward to your reply.

Sincerely, on behalf of the authors,

Daniel Roth

Review Comments and Responses

Reviewer #1:

1. This reviewer is of the opinion that the statements on the reason for choosing the dose of 70,000 IU vitamin D and the sample size calculation are important information to be added to the text.

RESPONSE: In accordance with this suggestion, we have added the dose and sample size justifications to the revised text.

Regarding the dose, we have added the following text to the manuscript on page 6:

“The dose was selected to be intermediate between the doses previously studied in the only two rigorous single-dose vitamin D3 pharmacokinetic studies published at the time our study was designed (50,0005 and 100,000 IU6), thus providing reassurance in terms of probable safety as well as enabling coherent between-study comparisons.”

And on page 10, we have added the following statement re sample size, which is a more precise explanation than we provided in our earlier response to this reviewer:

“The target sample size of at least 12 analyzable participants per group was originally justified as follows: assuming two samples per subject (baseline and peak), a standard deviation for the change in [25(OH)D] from baseline to peak of 20 nmol/L and an intra-
subject correlation of 0.6, we anticipated that at least 12 women in each group would enable the estimation of the mean $\Delta C_{\text{max}}$ with 95% confidence bounds of $\pm 10$ nmol/L.”

2. The references 5 and 6 referred to in response to comment 2 are in pregnant adults and not non-pregnant adults.

**RESPONSE:** Our previous comment included the statement that, “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$^1$ and 100,000 IU$^2$)”. The studies by Armas et al. and Ilahi et al. involved non-pregnant adult participants. We were not aware of any rigorous single-dose vitamin D3 pharmacokinetic studies in pregnant adults, and thus referred to these studies in non-pregnant adults as benchmark comparisons.


**Reviewer #2:**

No further comments.

**Reviewer #3:**

**Major Comments:**

1. The paper has been substantially improved in revision. I believe, however, that further shortening and clarification would be both possible and helpful. As just one example, the Discussion somewhat duplicates the Results. There appears to be some internal inconsistency in the presentation, which probably contributes to the length, and which, if cleared up, could both make the authors’ points better, and more economically. The authors stress in their Introduction and the Abstract that they focused on a “model-free” PK analysis, i.e., $C_{\text{max}}, T_{\text{max}}$, and AUC, principally. However on pages pp. 10–11 the focus seems to be on a specific model, and in the authors’ reply to my prior comment about discrepancies in the half-time values, the authors state that they used “three models” to compute the half-times. My recommendation is to concentrate on the foregoing PK parameters and on safety, and drop much or all of the rest. I was initially puzzled by the half-time issue, but focused too exclusively on the discrepancy in the numbers, when perhaps I should have paid more attention to how the half-time was actually calculated (which is not clear in the paper). Presumably it would have been derived from the slope of a log linear plot of the deltaD25 values after the peak. However, Ilahi and others did not find an exponential curve after a bolus dose, and inspection of Fig. 4 in this paper does not suggest an exponential drop-off. Instead, there is a nearly linear decline in deltaD25 with time. That is not terribly surprising because, despite the authors’ conclusion that a single compartment model works, that is probably not the case, inasmuch as continued, slow release from the fat depots of the native D that was not
immediately converted to D25 constitutes a continuing fresh infusion of the precursor for the 25-hydroxylase, thereby “propping up” the serum D25 concentration. In the final analysis I am not sure what a half-time estimate contributes in this context. Nor am I sure what it means. Half-time of what? D or D25? in what body compartment?

**RESPONSE:** Upon consideration of this reviewer's detailed comments and rationale (i.e., to avoid confusion and to further shorten the manuscript), we have decided to drop the secondary PK analysis entirely from the manuscript. Statements in the discussion that refer to this analysis have also been deleted, and one table and one figure were deleted. None of the major inferences/conclusions changed.

2. The authors make reference to some of the other papers describing D25 status in pregnancy, but completely omit the Hollis/Wagner paper [JBMR 2011;26(10)234-257], which is substantially larger than the current study (though it involved continuous moderately high dosing rather than a bolus dose). Hollis and Wagner were also concerned with safety, and provided a great deal of data to that end. At very least their findings need to be integrated with those of the present study.

**RESPONSE:** Our original rationale for not discussing the Hollis/Wager paper was because it was a daily dosing trial rather than a single-dose pharmacokinetic study. However, we acknowledge that this is an important prenatal vitamin D trial, and thus have added a paragraph about this study in the Discussion, on page 15.

Minor Comments

1. Page 8, bottom – Please give the units for the adjusted calcium algorithm.

**RESPONSE:** We interpreted this comment as referring to the sentence, “An albumin-adjusted serum calcium concentration >2.60 mmol/L prompted a repeat measurement on a new specimen as soon as possible”; the sentence was divided between pages 8 and 9 and the units “mmol/L” were at the top of page 9 (but the phrase now appears entirely on page 9).

2. Pages 12–13 – The authors state that the day 2 value was significantly lower in the pregnant group (which the graphs seem to support), but significantly higher at day 21, which is not apparent from inspection of the graphs, particularly Fig. 3. I do agree, however, that the overall shape of the graphs suggests slower 25-hydroxylation in the pregnant women.

**RESPONSE:** Figure 3 shows absolute [25(OH)D] and thus it may be difficult to visually perceive differences in delta-[25(OH)D] because of the different baseline [25(OH)D] of the two groups. The between-group comparison of delta-[25(OH)D] is shown in Table 1.

3. The term “deltaCavg28” is unclear, and might be best simply spelled out.

**RESPONSE:** The term is defined on page 10, as “An individual’s average Δ[25(OH)D] during the first 28 days (ΔCavg28).” We have now added this to the list of abbreviations as well as other similar abbreviations. However, we will defer to the editors and can write this out in full with each usage if that is preferable.
4. Page 18, line 3, et seq. – The authors state that references to the mechanism of toxicity were removed from the paper, but this one remains, and I still think that it is incorrect. According to Vieth, the toxicity is due primarily to displacement of 1,25D from the D-binding protein. For bolus doses, such as used in this study, there would not be time for a substantial rise in 25D. Interestingly, and consistent with some displacement of 1,25D from the D-binding protein, is the trend for a rise in serum calcium (though within normal limits) in the days following the single dose.

RESPONSE: We interpret this comment as referring to the following sentences (now on page 16 of the revised manuscript):

“An isolated serum [Ca] value above the reference range in one pregnant participant occurred in the early post-partum period, when albumin-adjusted [Ca] typically peaks [(18)]. This was not due to vitamin D toxicity because her [25(OH)D] at the time was 47 nmol/L and the [Ca] rapidly and spontaneously normalized.”


We agree with the reviewer’s comment about the likely mechanism of vitamin D toxicity as outlined by Vieth. However, the single above-range [Ca] value occurred on day 70 after vitamin D dosing, well beyond the period when the displacement of 1,25D from the DBP would be expected to be incurred, and not within the first days when we observed a average increase in [Ca]. We hope that with this clarification regarding the timing of this event (as shown in Table 4 and Figure 6), the reviewer will agree that the wording is acceptable.