Author's response to reviews

Title: Vitamin D Status of Older Adults of Diverse Ancestry Living in the Greater Toronto Area

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Author's response to reviews: see over
Dear Ms. Olino and Dr. Pioli,

Please find attached the revised manuscript: 8694380279529855 - *Vitamin D Status of Older Adults of Diverse Ancestry Living in the Greater Toronto Area*. We have considered all of Reviewer One’s (Robyn Lucas) comments in this revised manuscript. Below, we have addressed each of Reviewer One’s concerns, noting point-by-point how we have modified the manuscript to comply with her requests or explaining why we have not made the requested changes. Reviewer One’s requested revisions are in bold and our response is provided in regular font.

**Major compulsory revisions**

1. The paper needs to be made more concise, with removal of repetition, including citing the numbers in the text that are available in the Tables. The text can be used to interpret, rather than repeat, what is in the tables.
   - An effort has been made to make the text more concise by removing some data from the text that are available in the tables. In some cases data was kept in the text as we feel that this information is important for the reader. Some data comparisons for the three ancestral groups were kept in the text despite being available in the tables as these comparisons are a key feature of our study.

2. There is a lack of clarity in the timing of the blood-taking through the manuscript. The Background notes “wintertime”, the Methods Apr-May. This needs to be consistent and explicit, since it is so important to 25(OH)D levels and their interpretation.
   - The aim of the study was to capture the lowest vitamin D levels for the older adult sample. We have added the following statement to the ‘Data Collection’ section to clarify this apparent discrepancy:
     
     The data collection was done at the end of the winter in an effort to record the lowest (wintertime) 25(OH)D levels, as vitamin D levels are lowest in Canada during the winter months (November to March) when sufficient UVB is not available for cutaneous vitamin D synthesis.

3. Make the abstract and main text consistent in terms of the placement of the comparisons with other studies being part of the results or the discussion (I recommend as part of the Discussion).
   - Comparisons with other studies (young adult and older adult Canadians) were moved to the Discussion section of the text.

4. Biochemical analysis: there is some evidence that the location from which the punch is taken in the blood spot affects the results – were the punches taken in a consistent spot in each blood spot, or was any attention paid to this?
The following statement was added to the “Biochemical Analyses’ section on page 4 to clarify that the collection of blood from the blood spot cards by ZRT laboratories was conducted in a standardized fashion:

6-mm disks were punched from the middle of the pre-stamped blood spot area containing the dried blood spots (Wallac MultiPuncher) and reconstituted with 600 µl of deionized water.

5. The Bland Altman plot not only indicates a mean difference of 10nmol/L, but suggests that that difference increases with increasing 25(OH)D level. It is unfortunate that only 10 samples were compared by the two methods. The discrepancies for the absolute values are actually quite large – in Figure 1, a serum reading of 80nmol/L is around 110nmol/L from the blood spot. The whole analysis rests on the accuracy of these blood spot results – and there is not convincing evidence here, mainly due to the small number of samples tested by both methods, that the absolute values are accurate.

6. Concordance: only one blood spot was tested for concordance, with what appear to be quite disappointing results – a difference of 7.5nmol/L from the highest to the lowest.

In points 5 and 6, the reviewer raises some issues about the measurement of 25(OH)D values, focusing on the comparability of the blood spot LC-MS/MS method with the conventional serum-based LC-MS/MS method, and the concordance of the results.

First, we would like to indicate that the blood spot LC-MS/MS method has been extensively validated by the company ZRT. This information is available at: http://www.zrtlab.com/component/docman/cat_view/4-technical-bulletins?Itemid=

For 25-hydroxyvitamin D3, the intra-assay coefficient of variation ranged between 11.3% and 8.6% (depending on the concentration of the sample), and the inter-assay coefficient of variation ranged between 15% and 10%. These values are not very different from what has been described for other methods, including LC-MS/MS methods (for example, see Wallace et al., 2010. Steroids 75:477-488). Additionally, when comparing the results of the blood spot LC-MS/MS method with those obtained with serum samples, the coefficient of determination (R²) was 0.91: There was an excellent correlation of the blood spot vs. serum 25(OH)D levels.

In addition to the validation carried out by ZRT, we independently evaluated the performance of the method by 1/ checking the concordance with a serum-based method that we used in a previous study and 2/ checking the concordance of a sample characterized in triplicate. Again, we observed a very high correlation of the blood spot and serum values: in fact, the coefficient of determination is identical to the coefficient obtained by ZRT in their internal validation (R²=0.91), as shown in Figure 1. We also reported that, although both values are highly correlated, the blood spot method tends to give higher 25(OH)D values than the
serum-based method, with a mean difference around 10 nmol, and this was more evident in the samples with higher 25(OH)D concentrations. In our opinion, these are not unusual results given the analytical complexities involved in the measurement of this metabolite, and don’t compromise the reliability of the results, or the conclusions of the study. As an example, Carter (2011, Current Drug Targets 12:19-28), reported that the differences between the mean LC-MS/MS estimates obtained by laboratories participating in the DQEAS (Vitamin D External Quality Assessment Scheme) and the reference NIST values (also LC-MS/MS based) increased with 25(OH)D concentration, showing similar patterns to what we found in our data. The reviewer has chosen to focus on the only outlier present in our Figure, which could have resulted from a punctual problem in the extraction of vitamin D from the serum sample, ignoring the totality of the data, which shows a very strong correlation between the measures obtained by both methods, and relatively small differences for the majority of the samples. The reviewer also states that “only one blood spot was tested for concordance, with what appear to be quite disappointing results – a difference of 7.5nmol/L from the highest to the lowest”. We find this comment surprising. The results of the analyses of the sample analyzed in triplicate were 110, 115 and 117.5 nmol/L. This difference of 7.5 nmol/L is relatively small, and represents just 6.6% of the average value. For example, Carter described that most of the methods used to evaluate vitamin D in the DEQAS samples (including RIA, automated EIA, Roche, HPLC and LC-MS/MS) had a mean bias higher than 7% from the NIST LC-MS/MS assay.

In summary, we disagree with the interpretations of the reviewer, and believe that the blood spot LC-MS/MS method is accurate and reliable. It is important to note that a slight overestimation of the 25(OH)D concentrations of the samples at the higher end of the spectrum would not have any major consequence in the interpretation of our results. There is no evidence of strong bias in determination of 25(OH)D concentrations under 75 nmol/L or 50 nmol/L, which are the thresholds that are conventionally used to define vitamin D insufficiency.

7. Please provide the age breakdown of the population. All participants were >60 years, but more information is required, particularly in order to compare the results to other surveys of older adults.
- Details of the age breakdown of the sample are provided in Results: Sample Characteristics on page 5.

8. In the limitations section, discuss the effect of having a volunteer population, selected from a community organization, i.e. probably more mobile and healthier than the general older adult population of the region. The volunteers may have a particular interest in being involved in vitamin D research and this may account for the high proportion on supplementation. It is worth considering this, so that the reader can see that the finding of mean levels of >80nmol/L may not be generalisable to the older population of this region, it does not affect the association between higher levels of supplementation and less vitamin D deficiency.
- We feel that we had addressed the issue of our sample not being representative of the general older adult population living in the GTA because of their membership in a group that promotes healthy ageing. However, we have added the following statement to the discussion of the limitations of our study on page 10 to further clarify this point:

Members of the SOOAC are probably more mobile and healthier than the general older adult population in this region, on account of their membership in a community organization that promotes active, healthy aging. Furthermore, it is possible that the members of the SOOAC who volunteered to participate in our study may represent those members that are interested in healthy ageing, aware of the benefits of vitamin D and actively supplementing with vitamin D.

**Minor essential revisions**

9. Data collection: exposure to UVB was measured. How was this done, what was the specific information sought? Was this time outdoors, time in the sun (i.e. self-report), or actually measured exposure to this specific wavelength? It would be unlikely that participants could report their exposure to UVB.
- Participants were asked to self report on their weekly exposure to the sun (duration of exposure and areas of the body exposed) and travel to sunny destination in the three months prior to taking part in the study. The following statement was added to the discussion of UVB exposure on page 4:
  UVB exposure (self report time spent outdoors daily on average and travel; to sunny destinations).

10. Statistical analysis: p is usually <0.05, rather than #0.05
- does the reviewer mean that p is typically reported as <0.05 rather than p<0.001. We choose to continue to use p<0.001 to reflect a stronger finding of statistical significance.

11. There is quite a lot of repetitiveness in the results section. Page 5: “the only variable that showed a significant difference...” is followed by “The remaining variables did not differ significantly”. These say the same thing and the latter sentence could be deleted.
- as noted above in response to revision #1, an effort has been made to simplify the text and remove some of the repetition in the results section.

12. Comparison with previous study. This section and the next may well be better placed in the discussion. The similarities and particularly differences between the two studies need to be discussed in conjunction with these results, particularly those factors that may make a critical difference to the comparison. For example: the different time of the year for sampling (Apr-May here, compared to Jan-Feb for the younger group); different samples (well elderly attending a community group, so presumably healthy and outgoing; compared to University of Toronto students who may well be
largely indoor living with limited leisure time); different sex ratios. It is interesting to note the difference in supplement use, but they are also very non-comparable populations.
- Comparisons with previous Canadian studies have been moved to the ‘Discussion’ section.

13. No use appears to be made of the reporting of exposure to UVB.
- exposure to UVB was not considered among the exclusionary criteria. UVB exposure, in the form of travel to sunny destinations, was discussed in the discussion of the methodological differences between the young and older adult study. In this case, UVB exposure was not found to have any impact on 25(OH)D concentration.

14. Page 9: “25(OH)D levels in ....older adults.......are substantially higher...than a similar sample of young adults, and this can be explained by the higher vitamin D supplementation” – I would qualify this with “partly explained”, since there are so many differences between the samples (as noted above) that this is likely to be only one part of the explanation.
- in reorganizing the comparisons with other Canadian studies to the Discussion section, the above statement was removed. The current statement, which is at the end of then the last paragraph comparing the young and older adult studies states that the differences in vitamin D status are largely driven by higher supplementation among the older adult sample. This follows a discussion of the methodological differences between the two studies that may impact vitamin D status.

Discretionary revisions

15. Provide an exact p-value for the comparison between males and females in the abstract
- the exact p-value is provided

16. Provide the abbreviation for 25-hydroxyvitamin D in the last sentence of the first paragraph of the Background
- the abbreviation is provided

17. Page 4: “the option to receive their circulating 25(OH)D levels was provided”. This may be better written: “the option to receive the results of their...”
- the suggested rewording was adopted.

18. “A contingency table analysis...was statistically significant” This is a rather odd way of reporting that there was a significant difference in the proportion who were vitamin D deficient, according to any cut-off, in the <800IU/day compared to the #800IU/day supplement groups.
- The questionable statements was removed and the following statement has
been added in its place:

These differences are highly significant (Fisher’s exact test p-value <0.001).

19. In the comparisons with Canadian health surveys, it would be useful, in view of the known differences in different 25(OH)D assays, to note that assay type for the cited studies.
- we have added a sentence to report that the method used in the CHMS study was the LIASON 25-Hydroxyvitamin D Total assay. Unfortunately, this information is not available for Ioannidis’ study.

20. There is a call (page 10) for more “studies to explore the extent of differences in vitamin D supplementation between community dwelling older adults and long term care residents, and how this influences vitamin D status”. But there are already many studies showing the effect of supplementation on vitamin D status. It would be important to note that any studies examining the differences in prevalence of vitamin D supplementation use in community dwelling adults and long term care residents would need to use random samples of both, to ensure that the results were a valid reflection of what was occurring in the population.
- a statement about the need for more studies that are more representative of the older adult Canadian population has been added.

Thank you for considering our manuscript for publication in BMC Geriatrics. Please let us know if we failed to adequately address any of the reviewer’s concerns.

Best,

Dr. Jaime Ginter and Dr. Esteban Parra