Author’s response to reviews

Title: Trajectory of vitamin D status during pregnancy in relation to neonatal birth size and fetal survival: a prospective cohort study

Authors:

Linnea Bärebring (linnea.barebring@gu.se)

Maria Bullarbo (maria.bullarbo@regionhalland.se)

Anna Glantz (anna.glantz@vgregion.se)

Lena Hulthén (lena.hulthen@medfak.gu.se)

Joy Ellis (joy.ellis@vgregion.se)

Âse Jagner (ase.jagner@vgregion.se)

Inez Schoenmakers (I.Schoenmakers@uea.ac.uk)

Anna Winkvist (anna.winkvist@nutrition.gu.se)

Hanna Augustin (hanna.augustin@gu.se)

Version: 1 Date: 31 Mar 2017

Author’s response to reviews:

Reviewer 1:

Minor Concerns

There are subtle differences between numbers in the text and in Table 1

Thank you for pointing this out. This oversight has been amended in the text (page 8, lines 171-176).

Lines 214 to 216: This sentence may be more meaningful if the word 'adjusted' is changed to 'adjustment' or 'adjusting'.

The word adjusted has been changed to adjustment (page 11, 230).
Other Concerns

1. Women that terminated their pregnancies, were lost to follow-up or had multiple gestation were excluded from the analysis. Were these women different from those included in the analysis? What were the reasons for termination? Were the vitamin D levels in women excluded from analysis different at any time from those in the study?

As multifetal gestation is a risk factor for both preterm birth and small for gestational age, it was deemed appropriate to exclude these from the analyses. A small number of women (N=13) moved during pregnancy and were lost to follow up as most had left the country. Pregnancy termination was mostly due to fetal malformations or chromosomal abnormalities discovered at routine ultrasound examination (N=31). Vitamin D was not analyzed among the women who terminated the pregnancy or who were lost to follow up. This information is added to the methods section (page 5, lines 99-101).

2. Gestational age was determined by routine ultrasound in the second trimester. The sole exclusion criteria was pregnancy exceeding 16 weeks at inclusion. How accurate are the 2nd trimester ultrasound scans for dating pregnancies? If the women were recruited from gestational age 4 weeks, is there a reason the scans were not done earlier?

Gestational duration at inclusion was based on the date of the last menstrual period. If dating by routine ultrasound significantly altered the gestational age at inclusion, so that the pregnancy exceeded gestational week 16, the woman was excluded (only two women were excluded due to this). The ultrasound was offered as a part of routine antenatal care and the authors had no control over when this was performed. Dating of pregnancy has been clarified (page 5, lines 97-99) (page 13, lines 276-277).

3. The authors should speculate on the possible mechanism(s) by which vitamin D levels in late pregnancy and vitamin D status trajectory affect the pregnancy outcomes they studied.

A section on the possible reasons for the association has been added to the discussion (page 11, lines 232-237).

Reviewer 2:

"Trajectory of vitamin D status during pregnancy in relation to neonatal birth size and fetal survival: a prospective cohort study." The authors present an analysis of a prospective pregnancy
study conducted in 2013-2014 in Sweden. The women enrolled before 16 weeks of gestation and were followed for pregnancy outcomes that included pregnancy loss, preterm delivery, small-for-gestational age, and low birth weight. 25(OH)D was measured twice: before week 16 and after week 31. A small protective association was found for first trimester 25OHD and pregnancy loss. High 25OHD (\geq 100 \text{ nmol/l}) was associated with lower odds of SGA and LBW compared with 25OHD <30 \text{ nmol/l}. Women with a large increase in 25OHD from first to third trimester had the lowest odds of SGA, LBW, and preterm delivery. The results of the study are intriguing, but there are a few methodological issues that could be clarified to improve the paper.

Thank you for this thorough review. We have tried to accommodate the suggested improvements, and clarify any ambiguous information.

Abstract

1) Provide units for the T1 25OHD and pregnancy loss association. The OR of 0.99 is for what amount of increase in 25OHD?

This has been clarified by adding the word continuous to the sentence (page 2, line 37).

2) Consider adding gestational age range of pregnancy losses in lines 34-35.

We would like to accommodate this suggestion, but this information is unfortunately not available. Pregnancy loss is not determined by antenatal care but by the emergency gynecology ward and these medical records were not included in the ethical approval for the study. Data on pregnancy loss was based on self-report information by the women and gestational duration at fetal demise was not always provided. We have tried to clarify this in the paper.

3) Lines 38-39. For reference, it would be helpful to include the measure of 25OHD in ng/ml.

This information has been added (page 2, lines 38-40).

Methods

4) Please add Ns to the first paragraph.

This information has been added (page 4, lines 99-100).
5) Consider including a Figure to show the total enrollment in the study and how women were excluded from the analysis sample consistent with STROBE guidelines for reporting results (https://www.strobe-statement.org/fileadmin/Strobe/uploads/checklists/STROBE_checklist_v4_cohort.pdf).

A flow chart has been added (figure 1).


In the current paper, we have chosen to use logistic regression analysis to assess associations between vitamin D status and pregnancy loss. We did not choose to use a survival analysis like Cox regression, because time to event data was not considered reliable (as pregnancy loss is based on self-reported data and the gestational age at fetal demise was not available, as previously mentioned). However, we thought this comment very valuable. Therefore, gestational age at registration for antenatal care and thus enrollment in the study (estimated by last menstrual period because ultrasound is performed in mid-pregnancy), was added as a confounder to the logistic regression models regarding pregnancy loss. This did not change the results for the association between 25OHD and pregnancy loss. This was added to the methods section (page 8, lines 160-164) and discussion (page 13, line 268-269), and in table 3.

7) Related to the above, why were women who terminated their pregnancies excluded from the analysis of pregnancy loss? I believe methods have also been developed to account for this competing risk. (Reprod Toxicol. 2008 Sep;26(1):31-5. doi: 10.1016/j.reprotox.2008.06.006. Statistical methods for estimating the probability of spontaneous abortion in observational studies--analyzing pregnancies exposed to coumarin derivatives. Also see: Wilcox A, Fertility and Pregnancy: An epidemiologic perspective. Oxford University Press, 2010, 157-158.)

Most pregnancy terminations were due to chromosomal abnormalities or fetal malformations, caused by chance or higher maternal age. Some women chose to abort these pregnancies while others did not. Therefore, pregnancy termination was not considered associated with vitamin D status. Missed abortions, i.e. established fetal demise but no spontaneous abortion, that resulted in induced abortion were classified as pregnancy loss and not termination. As mentioned earlier, time to event-data was not available (as pregnancy loss is based on self-report data and the gestational age at fetal demise was not available). In addition, vitamin D status was not assessed
among women who terminated the pregnancy and there were only 31 such cases. This is mentioned in the discussion section (page 14, lines 281-282).

8) Line 103, "SGA was defined as weight or length at birth <2 SD…" I think the author's mean, ">2 SD," as in a birth that was MORE than 2 SD from the mean was considered SGA?

Thank you for spotting this mistake. This has been clarified.

9) It sounds like there are two different definitions of "SGA" one for weight and one for length? Is that correct, or were both short and light babies combined to make one category of "SGA"? Would the authors clarify this outcome?

In this paper, we only used the definition based on either weight or length. As it is common to define SGA by birth weight only, the rates of SGA were presented by both definitions. In order to avoid confusions, the SGA rate by birth weight only (i.e. the definition we did not use in this paper) was removed from table 1.

10) Was it possible for SGA pregnancies to also be preterm? I am not sure that the LBW outcome adds anything to this paper and could be removed, given the inclusion of SGA and preterm delivery.

Yes, it was possible for one birth to appear in all outcome categories (SGA, LBW and preterm delivery). The LBW outcome was included as an outcome, due to its high clinical relevance for future health. Preterm delivery is not necessarily a major determinant of future health, as it ranges from extremely premature children to those born almost at term. We agree that LBW provides similar data as preterm birth and SGA, but LBW might be the more extreme outcome of the three. In addition, LBW is poorly studied in relation to vitamin D status, despite being easy to assess as it does not require information on gestational age at delivery. In providing data on this outcome, we hope to provide data that could be easily tested in other settings (including countries with less access to antenatal and obstetrics care) and provide data on a clinically very relevant outcome.

11) Line 103-109. The authors describe their outcomes before defining how gestational age was estimated, it would help to have that information here.

This information has been moved to page 5, lines 97-98.
12) Also, how was gestational age estimated for miscarriages? The authors have medical record information which presumably includes gestational age for miscarriages?

As stated in the discussion section (page 13, lines 277-280), pregnancy loss was based on self-report data. Miscarriages are not diagnosed by antenatal or obstetrics care but by the emergency gynecology ward. We did not have ethical approval to access those medical records. It is also likely that early miscarriages were not always diagnosed or treated by the emergency gynecology ward, because the common advice is to wait until the bleeding subsides. To clarify how the data on pregnancy loss was collected, this information was added to the methods section (page 6, lines 113-115).

13) Line 114. The third trimester blood sample was drawn after gestational week 31, were any preterm births delivered prior to week 31?

Yes, there were a few (N=10) who delivered before the second blood sample could be drawn. This has been added to the results section (page 9, lines 181-182).

14) Would the authors explain the creation of the "season of conception" variable? It only includes two categories and appears to combine spring with winter and summer with fall? Rather than isolating summer (April to September)? (For example, see Lundqvist et al. PLoS One. 2016; 11(3), Vitamin D status during pregnancy: A longitudinal study in Swedish women from early pregnancy to seven months postpartum.)

Season was defined by the months that UVB-exposure is sufficient for endogenous 25OHD production in Sweden. This definition is also based on previous data from the current latitude in Sweden (Brembeck P. Determinants of vitamin D status in pregnant fair-skinned women in Sweden. Br J Nutr. 2013;110(5):856-64). This definition of season previously captured almost 30% of the variation in 25OHD concentration, among pregnant women on the current latitude (57-58°N) in Sweden. The study by Lundqvist et al was conducted on a higher latitude (64°N) which might warrant a different definition.

15) Lines 143 to 146. Please clarify the referent groups for each outcome. For example, was SGA compared with AGA alone or AGA and LGA combined?

This has been clarified (page 7, lines 150-152).
16) Line 144. Please define "change in 25OHD" is this a continuous value? Was T1 25OHD subtracted from T3 or the other way around?

As stated on page 7, line 145, T1 was subtracted from T3 and change was coded into 3 categories.

17) Line 149-151. "Tobacco use and vitamin D supplement use were also investigated as potential confounders but did not show any confounding effect..." I do not believe that vitamin D supplements themselves are in truth confounders, but rather a surrogate for the exposure (similar to an instrumental variable), thus they should not be adjusted for. However, I am confused by this statement? Can the authors hypothesize why supplement use is not a "confounder"? Vitamin D supplement use should be a good predictor of vitamin D status, and if vitamin D is related to the birth outcomes, I would expect supplement use to also be related? (Perhaps some of the text from the Discussion could be moved here to help explain this confusing finding?) It might also help to describe what was captured as a "supplement". Were these multivitamins? Prenatal vitamins? Cod liver oil? What were women in this study using as supplements? Supplements containing multiple vitamins/minerals could be confounders, thus the need for a bit more description.

Supplement might be considered as a confounder between vitamin D status and health outcomes, due to lifestyle and health awareness among supplement users. So, vitamin D intake could be a confounder due to other lifestyle factors it is associated with. In this case it could also be a confounder due to other nutrients in the supplement. The rate of supplement use and that most women used multivitamins has previously been published. This now clarified in the discussion (page 11, lines 228-229).

18) Line 152-153. The sentence "Correlation between T1..." is confusing as we are not told how change in 25OHD was defined in this analysis? Was it absolute value or did values range from a decrease over time to an increase over time? A negative correlation suggests that as T1 25OHD increased, the "change in 25OHD" decreased, but I'm not sure if that means the change in 25OHD is getting smaller, or more negative, and does negative mean a decrease over time?

The correlation coefficient is probably a reflection of the seasonality of vitamin D status during pregnancy: high T1 25OHD indicates sampling during summer or autumn which means T3 sampling was conducted in winter or spring- hence the negative correlation. This analysis was preformed to ensure that the variables could be included in the same model. This has been clarified (page 8, lines 159-160) and that the variables were continuous has also been clarified (page 8, line 159).
19) Line 155. "...due to the small number of cases." Consider adding the number of women/losses here.

This information has been added (page 8, line 166).

Results

20) Line 168. "...of which 37 were light and 56 short..." are the authors referring to SGA outcomes here? If so, it might help to use consistent language (i.e. "Of the SGA deliveries, 37 were SGA by weight and 56 were SGA by length").

Thank you for this suggestion! The sentence was changed according to the suggestion.

21) Lines 176-178. Are these "data not shown"? Please consider including the results of sensitivity analyses in a supplemental table.

These are data not shown, which has been clarified in the text (page 10, line 191 and 201). In excluding the preeclampsia cases from the models, the number of cases are reduced. Therefore, the models are underpowered, and some p-values change. The effect estimates are not very affected by this. We are therefore slightly reluctant to add this into a table, because we do not wish to put too much emphasis on these results. We are of course willing to add this information in a supplement, if the editor considers it important.

22) Lines 181-187. Please specify the comparison group for each of these results.

Information on reference category for change in 25OHD has been added (page 10, lines 193-199).

23) Tables. It would help to see the N's for the results tables. This could be done by including a column before each outcome with the Ns. Alternatively Table 1 could include columns for 25OHD. For example, in Table 3 it is unclear how many births were both SGA AND deficient in 25OHD? Also, be sure to clarify how many pregnancies were excluded from the multivariable model due to missing covariate information.

The total number (and %) of cases is available in table 1. The number of cases per 25OHD category has been added to table 2. Information on the number of cases in the models has been added to table 3.
Discussion

24) Lines 263-264. Was physical activity related to 25OHD status in the women with both measures?

Data on PAL was only available for about half the cohort and only those who could read in Swedish. Due to the large number of missing cases, and the biased ethnicity of the responders, this data was not used. However, PAL was not related to vitamin D status or to change in vitamin D status.