Author’s response to reviews

Title: Maternal Vitamin D Status, Gestational Diabetes and Infant Birth Size

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Version: 1 Date: 08 Sep 2017

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Editor Comments:

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Response: We thank the reviewers for the encouraging and valuable comments and suggestions. Hereby we provide point-by-point replies to these. We feel that the suggested modifications have substantially improved the manuscript.
Reviewer reports:

Alec Ekeroma (Reviewer 1): Congratulations on performing this study which adds to our knowledge of the associations of Vitamin D status and GDM.

The flow of the English grammar in the first paragraph needs revision.

Response: We have revised the first paragraph in the Background section (lines 59-68, page 4).

Vitamin D deficiency, defined as a circulating 25-hydroxy vitamin D (25(OH)D) concentration below 50 nmol/L, has been common among Finnish pregnant women [1]. However, due to recent changes in national health policies, intake of vitamin D has increased resulting in decreasing rates of vitamin D deficiency [2-5]. Vitamin D deficiency associates with gestational diabetes mellitus (GDM) [6], but the evidence is inconsistent [7, 8]. Of all pregnancies, 1-14% are affected by GDM [9], and globally GDM prevalence has been increasing in line with increasing obesity [10]. GDM is the most common pregnancy complication in Europe [11]. In Finland, the prevalence of GDM has increased from 5% in 2008 to 11% in 2014 [12]. GDM increases the risk of adverse pregnancy and neonatal outcomes, and the risk of obesity, metabolic syndrome, diabetes, and cardiovascular disease in later life both of the mother and the child [13].

The median is commonly used to measure central tendency in Vitamin D studies as the data is normally skewed but I am sure the authors have had this checked and found the median and mean similar.

Response: The means and medians are similar in both vitamin D concentrations. This information is now given in the Materials and methods section (lines 198-199, page 10). We chose to use the mean values because we have used statistical analysis ANCOVA, which tests and reports the mean values.

The GDM mothers were significantly VitD deficient at delivery compared to non-GDM and obesity as an association was acknowledged.

The only significant limitation of the study was that not all mothers had an OGTT and this was acknowledged. The advantage of this study is the homogeneity of the population sample which showed most are Vit. D sufficient and other factors such as genetics, lifestyle and diet which may affect birthweight are minimized.

Response: Thank you for these comments.
Of interest is the association of the UCB VitD with the HC and future studies need to mention whether the same midwife did the measurement and whether they were blinded.

Response: The study families were recruited to the cohort 1-2 days after delivery, if the inclusion criteria were met. Umbilical cord blood sampling and birth size measurements were performed at the maternity hospital, and midwives did not know which woman/family would be recruited or agreed to participate in the study. Birth size, including head circumference, was not measured by a single midwife.

The following sentence was revised in the Materials and methods section (lines 84-85, page 5): At Kätilöopisto Maternity Hospital, Helsinki, Finland, 987 families were recruited into the VIDI study between January 2013 and May 2014, after delivery during the mother's hospital stay.

In addition, a clarification was added in the Materials and methods section (lines 149-151, page 8): Birth size, including birth weight (kg), length (cm), and head circumference (cm), was measured by midwives according to standard procedure. These data and the duration of pregnancy were retrospectively collected from birth records.

Kathryn Hart (Reviewer 2):

Abstract:

Could the rationale for the interest in birth weight be clarified (if word limit allows) as there is no background given as to why this was chosen as a secondary outcome

Response: We revised the following sentence in the Abstract (lines 25-26, page 2): Background: Maternal vitamin D status has been associated with both gestational diabetes mellitus (GDM) and fetal growth restriction, however, the evidence is inconsistent.

The rationale for only studying those of normal weight is unclear (when you are interested in the relationship between Vitamin D and birth weight).

Response: There are several reports on associations between poor maternal vitamin D status and low birth weight. However, it has been suggested that birth weight and later disease risk has a U-shaped association, also within the normal birth-weight range (Harder et al., Birth Weight and Subsequent Risk of Type 2 Diabetes: A Meta-Analysis, Am J Epidemiology (2007), 165: 8: 849-
Thus, we wanted to explore whether, in our cohort of normal-birth-weight infants, an association exists between vitamin D status and birth weight.

We added a paragraph in the Background section (lines 69-71, page 4): Fetal growth may have later health implications also within the normal-birth-weight range [14]. Poor maternal vitamin D status has been related to fetal growth restriction [15] but it is unknown whether maternal vitamin D status associates with birth size in infants with normal birth weight.

Could you state prevalence of Vitamin D deficiency/ insufficiency in abstract too before you start looking at associations between GDM and Vit D?

Response: We added the following sentence to Abstract (lines 35-36, page 2): Of the study population, 97% were vitamin D sufficient [25(OH)D ≥50 nmol/L].

You state that both >80 and >50 are optimal Vit D levels for pregnancy. Whilst this may be explained in the main paper I think it would be useful to make it clear (in the abstract too) where these two recommendations arise from/ why they are different.

Response: We defined >80 nmol/l as optimal and >50 nmol/l as sufficient vitamin D status.

We have now clarified this in the Materials and methods section (lines 111-121, page 6): We defined vitamin D deficiency as 25(OH)D < 50 nmol/L, and vitamin D sufficiency as 25(OH)D ≥ 50 nmol/L, since a concentration of ≥ 50 nmol/L is considered sufficient for bone health [17]. Suboptimal vitamin D status was defined as 25(OH)D < 80 nmol/L, and optimal vitamin D status as 25(OH)D ≥ 80 nmol/L, as has been suggested based on calcium absorption studies [18].

However, unfortunately, we could not add these explanations to the Abstract because of word limitation.

Line 155 'The diagnosis of GDM WAS based on a two-hour 75 g oral… (missing word)

Response: We corrected the sentence in the Materials and methods section (line 156, page 8): The diagnosis of GDM was based on a two-hour 75 g oral glucose tolerance test (OGTT).
Line 166 'The normality of the variables was visually inspected. Outliers (n = 18) of 25(OH)D concentrations were omitted' - this does not sound very robust? How did you define your outliers? These should be defined statistically and the method for doing this clarified.

Response: Outliers were identified with Normal probability plot of residuals, and with Leverage and Cook’s Distance diagnostic tests. Outliers can be significant source of bias in the regression models, thus we excluded them from the analyses.

We modified the sentence in the Materials and methods section (lines 168-170, page 9): Outliers (n = 18) of 25(OH)D concentrations were identified with Normal probability plot of residuals, Leverage, and Cook’s Distance diagnostic tests, and omitted from the analyses.

Line 177 repeated measures ANOVA (missing word)

Response: We added a word in the Materials and methods section (lines 180-182, page 9): Independent sample t-tests, Mann-Whitney U-tests, repeated measures ANOVA or the Pearson Chi-Square test, when appropriate, were applied to compare maternal characteristics between GDM and non-GDM mothers.

Line 182 - prevalence of Vitamin D

Response: We corrected the sentence in the Materials and methods section (lines 186-187, page 10): Prevalence of vitamin D deficiency in non-GDM and GDM mothers were tested with Fisher’s Exact test.

Line 188 - ‘with Bonferroni correction when applicable adjusted for maternal length’ - what does 'length' mean? Do you mean weeks gestation or maternal height?

Response: We meant maternal height. We corrected this in the Materials and methods section (line 192-193, page 10), and in the Table 2 footnote (page 15): …Bonferroni correction when applicable, and adjusted for maternal height…. a Adjusted for maternal height and other listed maternal factors as changing covariates

Line 191 - 'in the models presented a slight problem regarding multicollinearity' - can you be more specific rather than 'slight problem'? How was this managed once identified?

Response: There was a multicollinearity problem in the analysis of GWG, however, this did not affect the results. ‘Slight’ problem was related to the fact that Q-Q plot and Leverage values
indicated that model was good, but Cook’s and Levene’s values/test indicated that there might be a problem of multicollinearity.

We edited the sentence in the Materials and methods section (lines 194-197, page 10): Using both 25(OH)D concentrations as covariates in the GWG analysis induced a multicollinearity problem based on Cook’s Distance and Levene’s test, but excluding these covariates from the model did not change the results.

Table 1 - p values for GWG values are missing
Response: We only tested the difference with repeated measures ANOVA, and since there was no difference between the groups (p=0.093, row 17), it was unnecessary to perform the post-hoc analyses.

Line 222 - needs rewriting 'Parallel in UCB, the adjusted analysis showed similar
223 25(OH)D concentrations in GDM and non-GDM mothers (79.1 ± 2.3 vs 80.1 ± 0.8 nmol/L, P = 224 0.69) (Fig. 1).' - presume you mean similarly in UCB… and then I would state these are the concentrations in the cord blood of infants born to women with/ without GDM (rather than 'in' the women directly)
Response: We edited the following sentence in the Results section (lines 229-231, page 12): Similarly, in UCB, the adjusted analysis showed no difference 25(OH)D concentrations in infants born to women with and without GDM (79.1 ± 2.3 vs 80.1 ± 0.8 nmol/L, P = 0.69) (Fig. 1).

Line 249 'As compared to mothers with suboptimal pregnancy 25(OH)D, mothers with optimal pregnancy 25(OH)D had heavier newborns (P = 0.010), but this positive association was not verified in linear regression (B 0.00; 95% CI -0.00, 0.01; P= 0.16). Newborn head circumference was larger in those with suboptimal UBC 25(OH)D compared with mothers with optimal UCB 25(OH)D (P = 0.003).' - are you presenting comparison with one vit D measure (preg) and one outcome (weight) and another (UCB) for the next outcome (head) because these were the only significant associations (e.g. pregnancy vit D was not sig associated with head circumference and UBC vit D was not sig associated with birthweight) or were these associations decided a priori?
Response: Comparing birth measures with vitamin D status was decided a priori, but linear regression analyses were added to confirm the significant associations between vitamin D status and birth size, and to gain more in-depth understanding.
Association between pregnancy 25(OH)D and OGTT results/birth weight, and between UCB 25(OH)D and head circumference were tested with univariate linear regression.

You are critical of the previous cross sectional studies yet the longitudinal nature of this study (two time points) is not exploited as there does not appear to be any analysis of change in Vit D status over time (and given the adequacy of Vit D levels in the sample this is unlikely to be informative) and all analysis is based on cross sectional associations. This is recognised in the discussion (‘However, in a cross-sectional setting causal relationships cannot be determined.’) so the criticism of cross sectional studies in the introduction is perhaps misplaced.

Response: Thank you for this comment. We have revised the following paragraph in the Background section (lines 72-75, page 4): Many of the findings regarding the relationship between vitamin D deficiency and GDM are based on case-control studies, which may include a potential selection bias. Case-control studies often focus on high-risk groups, for example women who are overweight and sedentary, which are independent risk factors for vitamin D deficiency as well (for example [16]).

Line 318 - could you discuss the clinical significance of the reduced head circumferences seen, i.e. were the differences seen clinically relevant? This is touched on in the conclusion but some discussion of possible mechanisms and consequences would be beneficial.

Response: Clinical relevance of the inverse association between UCB vitamin D status and head circumference at birth remains unsolved, although others have reported the same. We have considered multiple error sources (for example the mode of delivery), but these have not added to our understanding. Long-term consequences of this finding remain to be investigated.

We added the following text to the Discussion section (lines 322-329, page 17): However, the clinical relevance, if any, of the inverse relationship between maternal vitamin D status and head circumference at birth remains unexplained. It is unknown whether this reflects differences in brain size or in skull bones’ structure, and need to be explored in future studies. The mean difference in head circumference was 0.22 SD units between the groups with optimal and suboptimal UCB 25OHD. A possible explanation to this might be a U-shaped association between maternal vitamin D concentration and fetal outcomes in a population with sufficient vitamin D status. We have previously suggested the U-shaped association between 25(OH)D concentration and inflammatory biomarkers in cord blood [8].
Line 334 slight rephrasing 'A further limitation is that the OGTT was not performed on all mothers'

Response: We modified the sentence as follows in the Discussion section (lines 343-344, page 18): Further limitation is that the OGTT was not performed on all mothers, and a slight possibility exists that the actual prevalence of GDM might be underestimated.

In addition, there were comments in the manuscript by Alec Ekeroma:

Referring to a sentence: None of the pregnant women in our study received insulin therapy nor other regular medication, but mothers with GDM obtained dietary counselling at communal prenatal clinics [19]

- Question was: Not even Metformin? Unusual

Response: No, this was due to study inclusion criteria, as we included only mothers without regular medication (see Recruitment and study participants -paragraph).

Referring to a sentence: In agreement with our findings, others have discovered that mothers with higher vitamin D concentration have infants with smaller head circumference at birth [36, 37]

- Question was: Same person and blinded?

Response: Rogriguez et al. (36) do not report clearly if the measurements were done by the same person but they state that a nurse measured head circumference at the hospital ward within first 12 hour of life. This study was a population-based birth cohort. Hanieh et al. (37) do not report if the measurements were conducted by the same person, however the original study itself was a double-blind randomized trial about micronutrient supplementation.