Author’s response to reviews

Title: Vitamin D status and dental caries in healthy Swedish children

Authors:

Johanna Gyll (johanna.gyll@vll.se)
Karin Ridell (karin.ridell@mah.se)
Inger Öhlund (inger.ohlund@umu.se)
Pia Karlsland-Åkeson (pia.karlsland_akeson@med.lu.se)
Ingegerd Johansson (ingegerd.johansson@umu.se)
Pernilla Lif Holgerson (pernilla.lif@umu.se)

Version: 1 Date: 30 Oct 2017

Author’s response to reviews:

Response to the academic editor and reviewers of the manuscript

NUTJ-S-17-00277

“Vitamin D status and dental caries in healthy Swedish children”

The authors would like to thank the reviewers for their valuable suggestions that have led to an improved version of the manuscript "Vitamin D status and dental caries in healthy Swedish children". We have gone through each comment carefully, and list our answers and accomplished changes below. The reviewers have requested more in depth information on the basic study (DViSUM) in which the present dental study was nested. Unfortunately we cannot give more detailed information in order not to violate copyrights for the journals where results for the basic DViSUM study are published (refs 22 and 23).

Please, find our point-by-point responses to the comments raised by the three reviewers. A revised manuscript with revisions highlighted in yellow is enclosed. We have focused (restructured and shortened) the methods and results section as requested but restrict highlighting’s to edits that are direct responses to specific comments from the reviewers.
Reviewer #1:

TITLE: appropriate

ABSTRACT:

41% participation rate in this aspect of the broader study is low.

Response: We acknowledge this comment, but as a consequence of the 2-year period between the intervention and the dental visit and that the target age group, i.e. young parents and many in vocational training, is highly mobile, many had left the recruitment areas in the meantime. A few parents (n=12) would not come for time reasons, which is describe in the response below to reviewer 3.

A suggestion is to shorten the background section of the abstract and enhance the methods and results section of the abstract.

Response: We have shortened the introduction section, and expanded the methods section with information on how vitamin D status was estimated, definitions of the intervention groups (including that the lowest group was the control group), and clarified what the outcome variables were. We have also edited the information in the results section. Since the abstract was found long we have not added more information Lines 0-11, 14-15 and 19-22.

The reviewer was initially struck by the question of whether there was a difference in the vitamin D level of children who were part of this study (n=85) and those who did not participate in this dental component (n=121)?

Response: We have compared (and present) the vitamin D baseline/follow-up values for those who participated in the dental examination (n=85) and not (n=121) in the results section. Lines 215-217.
BACKGROUND:

Paragraph 3, line 59-60 - citations are required to support this statement.

Response: We have made the link between the text in the previous lines 59-60 clearer with the subsequent sentence where examples of conflicting results and supporting references are presented. Line 58.

Line 60-61 - the authors reference a study by Herzog which used data from the US NHANES. They could also consider reviewing Schroth, et al 2016 Vitamin D and dental caries in children J Dent Res, as data reported in that paper was based upon a representative sample of Canadian children 6-11 years of age which is similar to the ages of children in the present study.

Response: We have replaced the previous reference 14 to Schroth, et al 2016 as suggested by the reviewer.

Paragraph 4, line 73-80 this text is more appropriately suited for the methods section rather than the background section. Please merge this into the methods section.

Response: We have moved the text to the methods section as suggested. Lines 102-106.

METHODS:

How were the 85 children distributed between the three randomly assigned supplementation groupings in the parent study? It is important to understand this.

How did those who did not return and participate in this dental study (n=121) differ from those who did participate (n=85)? Were there differences in the mean 25(OH)D levels between these groups?

Response: Of the 85 children, 37 (42%) in the 25 µg per day group, 38 (45%) in the 10 µg per day group, and 10 (12%) were in the placebo group. We have added a flow diagram (Fig. 1) in response to reviewer 3 where this is described, and also in the text in the methods section. Lines 95-97.
For the second comment, please see answer above and added text in lines 215-217.

Line 125 - what is meant by medial upper and lower incisors? Do you mean central incisors?
Response: We have corrected the term as suggested throughout the manuscript.

Overall, while the methods are very detailed, they are extremely long - 6.5 pages. Please try to be succinct and reduce the amount of text. Alternatively, some of the methods could become a supplemental document to the manuscript.

Response: We have reduced the methods section substantially where the methods are fully described in the references. The descriptions of the serum/plasma analyses are also described in the cited reference but we have chosen to keep much of the text as it is key information in the study.

Regarding the logistic regression models, did the authors ever consider performing a backwards logistic model with all independent variables and then seeing which variables remained significant? Did the authors ever consider just including those variables that were significant or approaching significance with caries at the bivariate level?

Response: We have added results for the backward option. As this result supports an inverse association between vitamin D status and caries we revisited the PLS modelling and adopted a two step variant to reduce noise. This is described in the up-dated text. Lines 187-194.

Yes, we have considered the option to only include variables that were significant in univariate analyses but have been advised not to.

The reviewer is wondering why the second model included BMI z-scores as the rationale for this was not clearly presented.

Response: We interpret the comment as it refers to why BMI as such was included in the model. BMI was included since several studies demonstrate a positive association between BMI and caries status (also in Sweden). Further, BMI may serve as a proxy for higher energy/nutrient intake, which may be a potential confounder. If this is a misunderstanding and the reviewer refer to why z-scores per se were used, the response is that this is in accordance with WHO recommendations for the age group.
RESULTS:

Table 1 - were the baseline and post-intervention vitamin D levels of children in the DViSUM (n=206) different than those only in the dental study (n=85)? It appears that those who returned had higher baseline 25(OH)D levels - does it possibly mean that there are some differences between those who returned and those who didn't complete this dental study?

Response: We present data for the comparison between participants in the dental subgroup and the non-responders in lines 215-217 and hope this is satisfactory in relation to this aspect too.

Table 2

- is this table useful? Wouldn't it be appropriate to compare these characteristics to the baseline cohort of children (n=206) to show if/how they differ too? This table should also have a column for all 85 children in the present study focusing on dental caries.

Response: We have deleted the table.

Line 39 - Caries score - are the authors referring to dfs/DFS?

Response: Caries score refers to dfs/DFS. We have corrected the text here and throughout the paper.

Line 41 - what do the authors mean by mineralization disturbance? Do they mean enamel hypoplasia?

Response: The term “mineralization disturbance” was changed to “enamel defects” in line with the description in the methods section.

Page 15, line 270 - the authors mention mean intake of products with extrinsic sucrose. However, did they have information on the actual amount of sugar intake and frequency of intake?

Response: As described in the methods section, diet intake was recorded as self-reported intake frequencies of sugar and vitamin D containing foods/food aggregates. We have clarified the latter aspect in the methods section by adding “diet with focus on sugar and vitamin D containing foods/food aggregates” to the text. Line 110.
We have not used the information to calculate amounts of sugar or sucrose consumed per day as the questions do not allow for an estimate of energy intake and therefore intake cannot be properly standardized for energy intake. Thus, numbers given are exposure frequencies per day, which is also more influential for caries development than amounts of sugar per se.

We have also changed the term “extrinsic sugar” to “added sugar” for clarity. Line 223.

Line 275 and 276 - data is not shown? Please consider showing these data. The review would like to see the distribution of the 85 children in this study according to these 3 groups.

Response: The following text was added. “Of the 85 children, 37 (42%) in the 25 µg per day group, 38 (45%) in the 10 µg per day group, and 10 (12%) were in the placebo group, and compared to 42%, 39% and 19%, respectively, in the basic study [23].” Lines 95-97.

Page 16, line 287-292 - the reviewer is wondering whether this paragraph is needed in this paper as it really isn't related to the main objective of this paper?

Response: The paragraph has been deleted as suggested.

Considering that this paper is too long, the results section lacks focus. It would be better if the authors could stick to the main objective(s).

If 25(OH)D was measured at 6 years of age why are the authors trying to associate it with supplement intake 2 years later? This doesn't seem to make any sense and is beyond the scope of this paper.

Response: The result section has been shortened substantially and restructured to focus more clearly on the aim of the study.

Line 290 - weren't follow-up results also 3 months after the baseline phase at 6 years of age? Presenting data on the correlation between baseline and follow-up 25(OH)D seems beyond the scope of this paper.

Response: This section was deleted.
Page 16, line 293-297 - how is this section related to the primary objective of this study? This should be a separate paper likely linked to the full cohort of children. The reviewer finds that the results section is not focused on the primary association of interest.

Response: We have deleted this section as recommended by the reviewer.

Table 3 - Again, what is meant by mineralization disturbance?
Is it DFS only or a combined dfs/DFS?
Response: Please see the answer above that mineralization disturbance was changed to enamel defects. dfs/DFS was corrected as described above.

Table 4 - please show the entire models so that the reader can see all the independent variables included. In the present form, the reviewer is unable to comment on the appropriateness of the models.
Response: We have described the models in the footnotes of the new Table 3.

The authors should really focus on the first set of rows "all subjects, regardless of supplement intake at caries examination" and just include supplement intake in the model. The other two types of subgroup analyses leave the impression of data mining. The authors should revisit their models.
Response: We restricted the models to all children and included reported intake of vitamin D supplement in model 2.

Page 19, line 342 - do they mean central incisors instead of medial?
Response: Please see answer above describing that this was changed.

Line 348 - please report the data.
Why weren't enamel hypoplasia and LL37 also included in the regression model?
Response: We have kept dental caries and enamel defects as two independent outcomes. In our opinion, it would be appropriate to include mineralization defects in relation to caries.
progression but not caries prevalence. We have also added results from the PLS model where enamel defects was the dependent variable. The result support the univariate results that no association is seen, which likely reflects that virtually all parents in Sweden give their infants vitamin D drops and that the drops are free of charge.

Page 20, line 350-354 - Show the full model, not all the small models. It makes the reviewer think that you are data mining.

Response: We show the model in the footnote and have restricted the model to all children as recommended.

Page 20, line 363-370 - maybe the study wasn't powered to detect this difference in LL37 considering you only had 85 children? If LL37 is lower in children with lower vitamin D levels, then wouldn't you hypothesize that LL37 is lower in children with caries?

Response: We agree that power may well be a problem and accordingly have stated as a clear caveat in the discussion as well as evaluations in strata. We were surprised to find that LL37 was higher in caries affected children, and one biological explanation that we could think of is that disease associated microorganisms trigger expression, which we tell in the discussion (Line 337). However, the results need to be interpreted with caution and evaluated in a larger study. In response to these comments (and the one below) we have added a separate paragraph on strengths and weaknesses of the study and pointed at the power issue here and in the conclusion. Lines 355-363.

DISCUSSION:

Page 21, line 378-379 - please provide references.

Response: We have added two references (reference number 14 and 31). Line 303.

Line 380-382 - Schroth et al 2016 J Dent Res discusses issues of caries among 6-11 year olds and vitamin D status and that previous 25(OH)D status might have influenced the caries seen at the time of actual dental assessment. It might be useful to review their discussion section of that manuscript.
Response: This discussion is also present in our discussion and we have now linked it to the
discussion by Schrot et al. by adding ref 14 as well as in the conclusion. Line 303 and 364.

Page 23, lin3 416-417 - references are needed.

Response: Two references have been added (ref. numbers 38, Colombo et al., 2016 and 39,
Malcolm et al., 2014) as requested.

Line 421-422 - the authors may not have had sufficient statistical power to study this. Any need
to comment on this? Where is the discussion on limitations of this study? There certainly are
several limitations - small sample, linking data from different stages of the study, response bias
to questions, not having 25(OH)D status at age 8 years, etc.

Response: We have added a specific section on strengths as weaknesses of the study and also pointed to the power issue in the conclusion. Lines 355-363.

How do the findings from this study compare to the other national studies - Herzog et al,
Dudding, Schroth et al 2016 J Dent Res?

Response: With the edits recommended by the reviewer it is now clearer that the results confirm
to those by Schroth et al. We have described this in the conclusion paragraph. We have not
specifically compared to the other studies as Schroth is the largest study and targets children in
the same age group. Line 364.

REVIEWER #2:

1. No control group (healthy children without Vitamin D supplementation)

Response: We are sorry that it was unclear that the group with the lowest level of vitamin D
intake (2 µg/day) is a placebo/control group. This level represents the intake from regular milk
intake since it would not be ethically accepted to have a group with 0 intake. This is now
clarified in the abstract and methods section in the resubmitted version. Lines 11 and 91 and in
the new flow chart.
2. Too much data in the Result. The Tables should be more simplified & organised.

Response: We have deleted substantial parts in the Abstract, Methods and Results section in response to reviewer 1.

3. Needs language editing by a class A editing service

Response: The manuscript had been edited by a first and a senior editor at the Springer Nature author services before submission. This is one of the editing services that the journal recommends. We have returned the manuscript and had it edited once more before resubmission.

REVIEWER #3:

Although not a statistical issue, it would have been helpful for the reviewers and readers to present a flowchart of the study design. It was confusing to understand what was measured at different time points: (1) baseline/intervention - when children were 6 years old, (2) three months after intervention, (3) two years after intervention.

Response: We have added a figure (Fig. 1) illustrating the flow diagram Line 99.

General comments:

* Please present medians and interquartile ranges whenever presenting means and 95%CI for continuous variables in tables.

Response: The variables that were assessed are normally distributed in the general population except for the dietary measures. In our opinion, adding medians with a measure of variation makes the tables more complicated and we have been asked to simplify the tables. We therefore prefer to leave the central measures and measures of variations as they are. Please, see also answer below on dietary variables.

* If normality assumption is not met, one suggestion is to conduct nonparametric tests (i.e., Wilcoxon rank-sum test, Kruskal-Wallis tests) instead of the Student's t-test and ANOVA.

Response: Please see response above.
A few concerns regarding the use of PCA or PLS: maybe just a misunderstanding of the variables that were used to conduct PCA or PLS. Please provide more details about this analysis.

Response: As mentioned above we are now using a two-step method for PCA and PLS that includes 106 variables in the full x block and for PLS caries (yes/ no) or number of teeth with defect enamel as y. This results in a selection of variables that are important in explaining the variations in y, which then are used in the final model. This is described in lines 189 to 194. The variables in the final caries model can be seen in the new Fig. 2. This method is doable since the SIMCA+ software allows inclusion of different types of data and co-varying variables in the model simultaneously. We have been asked to reduce the paper over all so we abstain from describing all 106 variables in detail.

Please present results separately by intervention groups in table 1.

Response: Please, see answers above stating that detailed information from the basic cohort cannot be included in the present manuscript as it has been published and is under copyright protection.

Detailed comments:

1. Lines 93-94: It will be helpful to present the proportion of participating children (n=85) who were from Northern vs. Southern Sweden.

Response: The numbers of children at baseline by living region is added in the text. Line 86.

2. Line 95: It will be helpful to present the proportion of participating children (n=85) who were fair vs. darker skin types.

Response: The numbers of children at baseline by skin type is added in the text. Line 87.
3. Lines 98-100: It will be helpful to present all results separately by the three intervention groups as at first visit in DViSUM, children were randomly assigned to receive 2, 10, or 25 μg of vitamin D3 per day in a milk-based supplement for three months.

Response: Results for the basic DIVISUM study are fully described in the references 22 and 23. We cannot republish those data. In addition, the key for the present paper is the levels of vitamin D in serum and not the intervention per se.

4. Lines 103-106: "Of the 206 children who participated in DViSUM, only 41% consented to participate in an examination of their dental status […] The major reason for non-participation was that the children's caretakers had moved out of the catchment areas": It will be helpful to present all the reasons as some reasons may be related to the intervention program.

Response: We acknowledge the potential concern, but apart from that the parents of 12 children in Northern Sweden expressed that they could not come to an additional tooth examination for time reasons, the only reason was that they had moved from the area. Please see answer to reviewer I too.

5. Lines 182-183: Were all continuous variables normally distributed? Very often, dietary intake data are not normally distributed. One suggestion is to present the medians and interquartile ranges as well as the means and 95% CI.

Response: We share the experience with the reviewer that dietary measures are often not normally distributed, but more so for nutrients and food items that are more infrequently eaten, i.e. many mark zero intake, as is the case in longer FFQs targeting more narrow food/food groups. This is not the case here but there is a slight right skewed distribution so we have confirmed that none of the dietary variables differed by caries group in non-parametric testing. This was added to the manuscript (Lines 168-169). It is also our experience that medians are not optimal for presenting food frequencies and prefer to keep the mean values.
6. Lines 183-184: Normality assumption should be met when conducting Student's t-test or ANOVA. If this assumption is not met, one suggestion is to conduct Wilcoxon rank-sum test or Kruskal-Wallis test instead.

Response: We are fully aware of assumptions and limitations for the data analyses are fulfilled.

7. Lines 188-190: Data for caries, food intake and vitamin D at baseline and at follow-up (three-months or two-years after intervention?) are repeated measures. Thus, one suggestion is to conduct intra-class correlation coefficients with 95%CI instead of Spearman's correlation coefficients.

Response: This section was deleted in response to reviewer 1.

8. Lines 192-194: In table 4, was "vitamin D status" used as a stratifying variable instead of an independent variable, as stated in the sentence?

Response: In the new version of the table (by recommendation from reviewer 1), serum vitamin D levels was an independent variable, only.

9. Lines 198-200: It seems "number of teeth", "tooth brushing", and "presence or absence of S. mutans" were collected at two-years after intervention; whereas "father's and mother's education levels", and "region of residence" were collected at baseline. If so, please explain how model 1 took into account the fact that those variables were collected at different time points?

Response: Number of teeth", "tooth brushing", and "presence or absence of mutans streptococci were measured at the time caries was scored, living region was confirmed at that time since the child could be re-invited, but highest level of education was information from 2 years earlier. However, we believe it is unlikely the fathers and mothers have increased their education level substantially during these two years.
10. Line 202: Blood samples were also collected at three-months after intervention, which coincides with February-March 2013. Please confirm that those months are still considered to be in the winter season.
Answer: Yes, these months are defined as winter.

11. Lines 205-206: By "sensitivity analyses by region of residence, skin colour, and vitamin D supplementation at 8y", does this sentence mean stratification analyses?
Answer: Yes, but the sensitivity analyses are deleted in response to comments on power by reviewer 1.

12. Line 207: minor typo here, did the authors meant "multivariate principal components analysis (PCA)" instead of "multivariate partial least square analysis (PCA)" (as currently written in manuscript)
Response: Thanks for seeing this. We have corrected the mistyping (Line 187).

13. Lines 207-210: There are a few concerns here:

a. Is the objective of using PCA or PLS in this paper for dimensionality reduction? If yes, this objective was not clearly stated. In addition, PLS is used to find linear combinations of the variables to predict response variables linearly ... What were the response variables in PLS?
Answer: PCA and PLS regressions in SIMCA+ were used to search for clustering of subjects and variables associated with caries/enamel defects, respectively. The response variables were either caries status (yes/no) or number of teeth with defect enamel. These matters are clarified in the methods section. (Lines 187-189), and the explanatory and predictive capacity of the PLS model is added in the results section (Line 263-269).
b. Were all variables continuous (i.e., lifestyle measures, medical variables)? What did "lifestyle measures" refer to? Were they variables on diet intake and socio-economic conditions which were collected at baseline? Same questions about "medical variables": did they refer to dental health-related behaviours which were collected at two-years after intervention? If so, there may be a mixture of, was this taken into account when conducting PCA and PLS?

Response: The SIMCA+ software used for PCA/PLS can handle continuous, categorical and binary variables in the same model as was the case here. Further, covariation is allowed between x-variables, which was also the case here and the software scales the variables and transformation is done if needed to normalize distributions. Please see answer above for variable definition.

c. Were PCA and PLS regression conducted on variables that were repeated measures (i.e., serum/plasma samples were collected at baseline, at three-months after intervention and at two-years after intervention) and on variables which were collected at different time points (i.e., lifestyle measures collected at baseline; medical variables collected at two-years after intervention)? If so, conducting PCA or PLS on such data is complicated as there are correlations among the same variables collected at different time points, and not all variables were collected at all three time points. In general, PCA or PLS are conducted on independent observations, thus I would question the use of PCA or PLS with repeated measures (i.e., longitudinal data).

Response: No repeated measures were not used in the PCA/PLS models.

14. Lines 226-231: Since children were randomly assigned to three intervention groups at baseline, one suggestion is to present the results separately by intervention groups. Especially regarding the mean vitamin levels, as these may be different depending on which intervention group the children belong.

Response: Since the reviewer mentions vitamin levels, that were only measured at DIVISUM before and after intervention, we assume the comment refers to those data. As told above those data are published and cannot be included here.
15. Lines 291-292: Was this Spearman's correlation coefficient? If yes, data for serum vitamin D levels at baseline and at follow-up (three-months or two-years after intervention?) are repeated measures. Thus, one suggestion is to conduct intra-class correlation coefficients with 95%CI instead of Spearman's correlation coefficient.

Response: This section was deleted in response to reviewer 1.

16. Table 1: It will be helpful to present results separately by intervention groups.

Response: Please see previous answers where we explain that we are limited in what data we can present for the basic DIVISUm study.

17. Table 3: 47 (caries-free) + 36 (caries) = 83, shouldn't this total be 85?

Response: We appreciate this comment. We have corrected the typing error (48 caries free and 37 caries).

18. Table 4: minor typos here, did the authors mean "Odds Ratios" instead of "β-coefficient"? In addition, did the authors mean "3 months after intervention" instead of "2 months after intervention"?

Response: We have changed this to Odds ratio and corrected to 3 months after intervention.

19. Table 4: It will be helpful to present the sample sizes for each “vitamin D status” because there are at least 5 variables included in model 1, 7 variables included in model 2 and 8 variables included in model 3 (this is to assess the statistical power when conducting logistic regression models using the rule of thumb of “at least 10 people per variable”).

Response: The data presented in this table are now restricted to all children as recommended by reviewer 1. Please see new Fig 1 (flow diagram) for numbers.