Author's response to reviews

Title: Intakes of calcium, vitamin D, and dairy servings and dental plaque in older Danish adults

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Author's response to reviews: see over
We would like to thank the reviewers of our paper for the comments that helped improve our original manuscript. Here is a list of the changes we made according to the reviewers’ suggestions.

**Reviewer #1:**

1. The authors acknowledge this is an observational study and make realistic conclusions at the end of the paper. However, the conclusion in the abstract needs to highlight the nature of the study as does the aim.

   Thanks for your comment. We agree that the conclusion might need a word of caution. We have now highlighted the cross-sectional nature of the study in the conclusion.

   “Conclusion: Intakes of calcium dairy-servings within-recommendations seem to be inversely associated with plaque, among those with higher, but not lower, vitamin D intakes. Due to the cross-sectional nature of the study, it is not possible to infer that this association is causal.”

2. It is not clear whether the sample was a convenience sample or whether it included all older adults from the Heart Study (subject to being mobile etc). This needs to be clarified.

   This was NOT a convenience sample. Among the subjects from the third follow-up of the Copenhagen City Heart Study who volunteered to continue in the current project, 1918 individuals, community-dwelling, aged 65 years or older identified as still living in Copenhagen were invited to participate in the present oral health study (COHSS) in 2004/05. In total, 783 of these individuals agreed to participate (participation rate: 40%). You can find this information on Figure 1. Due to limited length for short-reports (1000-1500 words) we had condensed the method section but we agree that this information was not completely clear in the text and therefore, we have clarified this point in the revised manuscript.

3. In the methods section there is no mention of the approach to intra examiner effects and whether these were accounted for - this needs to be remedied.

   Thanks for raising an important methodological point. One dentist (KH) trained by an experienced clinical examiner (PH-P) assessed the presence of visible detectible plaque by visual inspection. KH was calibrated before, during and after the clinical examination period against PH-P.

   Unfortunately, inter and intra-examiner kappa for plaque was not calculated, however plaque was measured by a very simple method (“present or not present”, an observation very easy for an experienced dentist, and it is therefore considered a robust variable. Furthermore, the inter and intra-examiner kappa for caries was calculated and showed a satisfactory result at all-time points during the 1.5-year data collection period - for inter-rater reliability, the Kappa-value was 0.98 in the beginning of the data collection period and 0.94 half-way through the data collection.
Furthermore, the Kappa-value for the intra-rater reliability was 0.97, which may be considered as excellent agreement.

Since caries assessment is more complicated and complex than visual inspection of plaque, and the validation study for caries showed good agreement between and within examiners, there is no strong reason to assume that the study was not significantly affected by intra-examiner effects. This section has been rewritten. We have also acknowledged the lack of intra-rater reliability for plaque score as a limitation of our study in the discussion section.

4. **Also the reference for the dietary reference values appears to be incomplete (ref 16). Are these recommendations country specific?**

We apologize for this mistake and the reference has been corrected, this reference being the recommendation by the American Institute of Medicine for general population (specific for each age group: children, adults, elderly and pregnant women).

5. **You refer to dairy servings - what does this actually mean - could you give a couple of examples - did you relate your classifications to overall percentage contribution of dairy foods to the Danish diet?**

The servings refer to a cup of milk; 1 slice of cheese (25-30g) and 1 pot of yogurt (150-180 ml). This information has now been added to the text.

We did not relate dairy servings classification to the overall % contribution of dairy foods to the Danish diet. The classification was based on the Food pyramid recommendation (the reference is stated in the manuscript text).

In our previous publication, using data on a sub-set of the study population, we reported that dairy foods were the major source of dietary calcium, accounting for 56% of total calcium intake. Milk was the major source of dairy consumption followed by fermented foods, cheese and other types dairy food (including cream, desserts, etc), respectively. Due to the lack of space we decided not to repeat these results. We have now mentioned these findings and included the reference to our recent published paper.

Amanda R. A. Adegboye, Lisa B. Christensen, Poul Holm-Pedersen, Kirsten Avlund, Barbara J. Boucher and Berit L. Heitmann

**Article:** Intake of Dairy Products in Relation to Periodontitis in Older Danish Adults

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6. **In the results section 96+78+606 add up to 780 - you refer to 783 participants - what happened to the other 3?**
We apologize for this misunderstanding. We mention that out of the total 783 participants, we had to exclude 96 due to edentulous condition plus 78 due to lack of information on covariates. So, we end up with a study population of 606 individuals and the revised text reads as follows:

“Of the 783 participants, 96 were edentulous, 78 did not complete the questionnaires, leaving a study population of 606 partial or fully dentate participants with complete information on diet, plaque score and covariates.”

7. The last paragraph of the results is one sentence - I would suggest you break it up into two - I needed to read it a few times to get the point.

We agree that breaking the paragraph [originally compressed to shorten the MS] to improve readability of the text.

Reviewer #2:

1. Please provide more information on previous observational findings related to calcium, vitamin D, dairy products, and dental health. What is the knowledge gap that leads to the present study (other than the subgroup analysis according to vitamin D intake)?

We appreciate your comment. This is short communication and due to space limit we could not write more about the previous findings in the literature in the introduction section.

Surprisingly, few studies have investigated the importance of interactions between dietary nutrients or effects of dietary patterns or specific foods (apart from sugar and caries) on dental health. Only recently, attention has been given to the associations between dairy products and dental health. As stated in the manuscript, most of the previous studies have not adjusted for vitamin D intake and none that we know of have stratified their analyses for vitamin D intake. Thus, to the best of our knowledge, this is the first observational study examining associations between calcium and dairy-servings intakes and plaque score (this has been stated in the discussion section).

2. In the section of dietary assessment, please provide sources (food or supplement) of calcium, dairy products, and vitamin D. Also, please move description of vitamin D from covariates to this section.

The description of vitamin D intake has been moved to the section on dietary assessment. For the analyses, we considered both dietary and supplemental intakes, Due to limited information on calcium content of the supplements used, 800 mg was added to the total amount of calcium intake, but only for those who reported taking calcium supplements daily. Supplements contributed to 30% and 48% of the average consumption of vitamin D and calcium, respectively. Detailed data on source of calcium (total dietary calcium, dairy-calcium and non-dairy calcium) have been presented in our previous publication. We now stated in the manuscript text that both supplemental and dietary sources were considered in the present analysis.
3. Analyses are not complete without first reporting the overall association. Suggest doing the following analyses and present these results in a new table:

Thanks for your comment. Since we found that associations were only statistically significant in the group with higher vitamin D intakes, showing the overall association could be misleading as the effect of the associations of dairy and calcium is diluted when combining both groups (high & low vitamin D intake). As expected the overall association is somewhere in between the associations described by high and low vitamin D intakes showed in figure 2. For dairy servings, OR = 0.71; p = 0.057; 95% CI= 0.51-1.0. For calcium, OR = 0.72; p = 0.098; 95% CI= 0.49 – 1.06). Due to the limited word allowance, we have omitted this information since the suggested analysis would significantly increase the length of the manuscript. However, if the editor judges this to be necessary, we can replace figure 2 with the suggested table.

4. For subgroup analysis, please also check whether the overall association may be modified by gender, alcohol intake, number of teeth, and No. use of dental floss. The results need to be provided in the results section.

This is an interesting point. We initially performed subgroup analyses by gender, age and education and we did not find any significant associations. As suggested, we have now stratified by alcohol intake but did not find any effect between groups. The analysis considering number of teeth is difficult since there is no recommended cutoff point to use to define groups. We have, therefore, dichotomized the ‘number of remaining teeth’ into >= 20 vs. < 20 teeth since there is evidence to suggest that 20 is the critical number of natural teeth necessary to maintain good eating and chewing function. Again, we did not find any significant difference. The number of individuals using dental floss was very low and subgroup analysis could not be performed. We have added this information from subgroup analyses to the results section.

5. The mean intake of vitamin D (<300 IU) is well below the recommended amount of 600-800 IU. The authors reported only 4% of the subjects had sufficient vitamin D intake. What is the clinical relevance for the present findings by vitamin D stratification, given that the majority of subjects did not have sufficient vitamin D?

Thanks for your comment on the clinical relevance of our findings. However, due to the data being cross-sectional and the lack of detailed information on supplemental intake of vitamin D, we feel that any clinical advisory statement would be premature. However, our results indicate that dairy foods and calcium intake seem to be inversely associated with plaque score, among those with higher, but not lower, vitamin D intakes, though this needs to be confirmed in further studies. Although dairy food (in particularly milk) is one of the more commonly eaten foods it has been the subject of remarkably little clinical research concerning its influence on oral disease. Therefore, our findings should be used to encourage further prospective studies on this particular foodstuff. Further studies on higher intakes of vitamin D, such as those recommended by the IOM, or higher are awaited in order to determine optimal vitamin D intakes for dental health.
6. In the discussion section, please provide more details comparing previous studies with the current findings. What are the similarities and discrepancies?

As initially stated in the discussion, this is the first observational study examining associations between calcium and dairy-servings intakes and plaque score. Thus, it is difficult to make useful comparisons of our results with other work. We state in the discussion that there is consistent evidence suggesting that higher intakes of calcium, dairy-foods and vitamin D are inversely associated with caries and periodontitis, though further data on dairy-food consumption and oral health, stratified by vitamin D intake is necessary.