Author's response to reviews

Title: Dairy products and calcium intake during pregnancy and dental caries in children

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Author's response to reviews: see over
Dear Dr. Gabriel:

Thank you for your email of February 17, 2012. We are pleased that you are interested in our manuscript for possible publication as an Original Article in *Nutrition Journal*. We appreciate the thoroughness with which the reviewers have considered our manuscript. We have addressed the comments raised by the reviewers and have carefully revised the manuscript.

I am sending the revised manuscript with changes highlighted in red.

Specific revisions and responses to each reviewer are described below.

We thank you in advance for your consideration.

Yours sincerely,

Keiko Tanaka, DDS, PhD
Reviewer 1
Dr. Ingegerd Johansson

The present study aimed at investigating the effect of maternal intake of calcium and dairy products and caries development in the deciduous teeth of the offspring. For this 315 mother-child pairs were included and followed from recruitment during pregnancy until between 41 and 49 weeks postpartum. Food intake was monitored in the mothers as were a set of potential confounders. The authors came to the conclusion that mother’s intake of cheese and yoghurt reduces caries development in early childhood.

Response:
We appreciate your careful review and helpful comments. Our revisions and responses are described below.

The research question is interesting and relevant to the field of Cariology and especially early childhood caries (ECC). However, the conclusions drawn by the authors are unfortunately not justified by the information provided in their paper. The reason for this is that caries is scored when the children are 3-4 years of age and the dietary habits of the child are not accounted for. This was a surprising finding as on the one hand the authors are aware of the issue (brought up in the discussion) and they have recently published a paper on dairy intake and caries in small children, and on the other hand because it would have been very easy to record when children/parents were seen for caries scoring. The conclusions in the paper must be considered premature unless the authors can add information that excludes that dairy intake tertile-classification of parent reflect the same tertile classification of the children. In contrary, it is likely that the maternal and child habits are parallel.

Response:
At the fifth survey, information on the dietary habits of the child over the previous month was reported. The mother of each child was asked to state how frequently her child consumed each of 51 selected food and non-alcoholic beverage items. In calculating intake frequency, total dairy products intake of children was defined as the sum of milk, yogurt, and cheese intake. The Pearson’s correlation coefficient between maternal intake of energy-adjusted total dairy products during pregnancy and children’s frequency of total dairy products intake was 0.26 (95% confidence interval [CI]: 0.15-0.36). The Pearson’s correlation coefficients between maternal intake of
energy-adjusted milk, yogurt, and cheese during pregnancy and children’s intake
frequency of milk, yogurt, and cheese at fifth survey were 0.22 (95% CI: 0.11-0.32),
0.16 (95% CI: 0.05-0.26), and 0.08 (95% CI: -0.03-0.19), respectively. Thus, maternal
dairy products intake during pregnancy is likely to have little relation to the children’s
frequency dairy products intake.

In the revised version, we added the children’s total dairy products intake frequency
as one of the a priori confounding factors in the multivariate models. The results
obtained in the re-analyses were nearly identical to those obtained in the original
analyses. We changed the estimates of odds ratios, 95% confidence intervals, and p
values for trend in the Results section and in Table 3 to reflect the results of the
re-analyses. We also added the following passages to the Methods section:

“In the fifth survey, each participant filled out a set of two self-administered
questionnaires. One of the self-administered questionnaires was a brief diet
history questionnaire that assessed the dietary habits of the child over the previous
month. Mothers of children were asked to state how frequently their children
consumed each of 51 selected food and non-alcoholic beverage items. Total dairy
products intake by children was defined as the sum of milk, yogurt, and cheese
intake.” (page 7, lines 7-13 in the revised manuscript)

“and children’s total dairy products intake frequency” (page 7, line 27-page 8, line
1 in the revised manuscript)

In addition, to this major problem there are some less, but still, serious issues that
need to be called into question.

1. The participation rate was alarmingly low in the study, 17% in the first
recruitment area and unreported for the second area. How have the authors dealt
with a possible recruitment bias, what is the evidence for or against such a bias,
and is this of importance for data inference?

Response:
As we had already mentioned in the Discussion section (page 12, lines 16-22 in the
revised manuscript), we recognize that the low participation rate at the baseline survey
was one of the weaknesses of the current study. We had also noted that educational
levels were higher among the parents in our study than in the general population. This
could have affected the results. Nevertheless, we were not able to evaluate the effect of
a possible recruitment bias on our results (over-estimation or under-estimation), because
information on personal characteristics, such as age, dietary habits, and oral health status, were not available for the non-participants in the OMCHS. Therefore, we are not able to add a more detailed description of the effects of selection bias on the results of the present study. As we had mentioned in the text (page 12, lines 22-25 in the revised manuscript), the prevalence of dental caries in our study subjects (23.5%) was lower than that in a sample of 4-year-old Japanese children in a survey of dental diseases in 2005 (44.2%). On the other hand, calcium intake in this study population is similar to that in the general population. According to the National Health and Nutrition Survey in Japan, the average daily per capita intake of calcium was 531.1mg, whereas the mean daily intake among our study subjects was 556.1mg. To communicate this, we added the following passage to the Discussion section:

“With regard to dietary intake, calcium intake in this study population is similar to that in the general population. According to the National Health and Nutrition Survey in Japan, the average daily per capita intake of calcium was 531.1mg [27], whereas the mean daily intake of our study subjects was 556.1mg.” (page 12, line 27-page 13, line 4 in the revised manuscript)

There are a few possible reasons for the low participation rate in the present study. In Japan, epidemiological studies are not conducted very often. Therefore, people are not familiar with epidemiological studies and are unlikely to be interested in them. In addition, our very low participation rate might also be attributed to the large size of our questionnaire (the lifestyle and health status and diet history questionnaires at baseline survey were 13 and 15 pages, respectively). We did not mention this circumstance in the text.

2. At inclusion women were between 5 to 39 weeks pregnant. This means they were between very early pregnancy and days before labour. Thus, baseline is at very different time points in relation to formation of the deciduous teeth, and also for some women the 12-month back tracking of the diet registration means that a year before pregnancy was monitored and for others that the pregnancy period was captured. It is questionable if this can be accounted for?

Response:
In the baseline survey, we used a semi-quantitative, comprehensive diet history questionnaire (DHQ) that assessed dietary habits over the previous month (page 6, lines 14-16 in the revised manuscript). Hence, almost all of our study subjects reported their dietary habits during pregnancy. Certainly, gestation duration among our subjects
had a wide range (5th to 39th week of pregnancy). As we had mentioned in the Discussion section (page 12, lines 12-13 in the revised manuscript), however, Cucó et al. [23] have shown that dietary patterns remain fairly constant throughout pregnancy. Therefore, food intake information obtained at one point during pregnancy is likely to reliably represent food intake throughout pregnancy. We added the sentence “Therefore, food intake information obtained at one point during pregnancy is likely to provide reliable information of dietary pattern throughout pregnancy.” to the Discussion section (page 12, lines 13-14 in the revised manuscript).

3. Reported mean daily intake of calcium was 556 mg/day, which is close to half of what is reported and recommended in Western countries without taking lactation into account. Is the low (recorded) intake a result of true low intake in this population or a result of underreporting in the diet recordings. Correlation coefficients (reported to be 0.51 versus weighed intake measures) reflect relative validity of subjects ranking and not of absolute intake levels. This is important to distinguish to be able to evaluate the results and their generalizability for other populations with a higher intake and presumably “better” status. Taking this into consideration, means and limits for tertiles (used for OR) should be presented for calcium intake at least.

Response:
According to the National Health and Nutrition Survey in Japan, the average daily per capita intake of calcium was 531.1mg, whereas the mean daily intake of our study subjects was 556.1mg. Thus the low intake of calcium reported by our subjects compared with that typical in Western countries is not an artifact of underreporting; rather, it represents the actual calcium intake that is typical among Japanese. As mentioned in the response above, we added the following sentence to the Discussion section:

“With regard to dietary intake, calcium intake in this study population is similar to that in the general population. According to the National Health and Nutrition Survey in Japan, the average daily per capita intake of calcium was 531.1mg [27], whereas the mean daily intake of our study subjects was 556.1mg.” (page 12, line 27-page 13, line 4 in the revised manuscript)

As you suggested, we added the range of dairy products intake and calcium intake for each tertile to Table 3. Elsewhere in the same table, we decided to use the medians rather than the means as a measure of central tendency. Median values seem to be used
more commonly in epidemiological studies on nutrition. For your reference, in the present study, the mean values of calcium intake by tertiles, from lowest to highest, were 390.8mg, 541.0mg, and 736.5mg, respectively.

4. The “Subjects and methods” section is unclear on several points and needs a careful revision. There are to many places where the reader has anticipate/guess, which is not appropriate. Some examples are:
(a) it is stated that that tooth status was recorded as sound, decayed or filled tooth. was a missing tooth not scored, or were there no missing teeth. If so this should be stated. How were sealants scored? The authors are recommended to introduce and use the dmft index for clarity, and avoid expressions as like “the mean number of dental caries per child was 0.87” since it is unclear what it refers to.

Response:
In the present study, the reasons for missing teeth were not identified. Therefore, children were classified as having dental caries if one or more primary teeth had decayed or been filled. We added the following passage to the Methods section: “The reasons for missing teeth were not identified in the OMCHS.” (page 6, line 4 in the revised manuscript)

Regarding the description of dental caries status, we modified the Methods section and the Results section as follows:

The sentence in the original manuscript “Oral measurements included the total number of teeth present, and the status of each tooth as sound, decayed, or filled.” (page 6, lines 2-3 in the original manuscript) was changed to “The number of dental caries was recorded as the number of decayed or filled primary teeth (dft).” (page 6, lines 3-4 in the revised manuscript)

The sentence in the original manuscript “The mean number of dental caries per child was 0.87.” (page 8, lines 5-6 in the original manuscript) was changed to “The mean number of decayed or filled primary teeth (dft) was 0.87.” (page 8, lines 24-25 in the revised manuscript)

(b) the term baseline survey must be clearly defined when it appears the first time in the text. At present it is not fully clear until the tables are read.

Response:
To clarify the term “baseline survey”, we added the phrase “which was conducted during pregnancy” (page 5, line 8 in the revised manuscript).

(c) it must be clearly stated what information was for the mother and what was for the child, e.g. tooth brushing frequency.

**Response:**
In order to clarify this matter, we added the words “in the child” in the Methods section (page 7, lines 7, 14, and 25 in the revised manuscript). We also added the words “in children” to Table 1 and the footnote of Table 3.
Reviewer 2  
Dr. Carlos Camargo

Tanaka and colleagues analyzed data from 315 mother-child pairs in the Osaka Maternal and Child Health Study (n=1002 women). The objective of this secondary analysis was to investigate the association between maternal intake of dairy products and calcium during pregnancy and the risk of childhood caries by age ~4 years. The authors report that higher maternal intake of cheese during pregnancy was associated with lower risk of childhood caries, while maternal milk intake was not.

Response:
We appreciate your careful review and helpful comments. Our revisions and responses are described below.

MAJOR COMPULSORY REVISIONS
1. Although the prospective design is good, the data are problematic in that only 494 mother-child pairs completed survey to age ~4 years, of whom only 315 were included in this analysis (ie, 31% of original sample). The authors should present actual data to support the comments in Discussion about differences between the larger starting sample (n=1002) and the sample use in the present analysis. Typically this is done at the start of the Results section and, given the number of factors, it would help to create a new Table for this purpose.

Response:
Thank you for your useful comments. We moved the description of the differences in characteristics between the 687 children who did not receive oral examinations or who were excluded due to incomplete information or non-participation in follow-up surveys and the study sample (n=315) from the Discussion section (page 11, lines 12-21 in the original manuscript) to the beginning of the Results section (page 8, lines 14-22 in the revised manuscript). With regard to the issue of creating a new table comparing the two groups, that is, the 315 included subjects and the 687 excluded subjects, we feel that this would be prohibitively difficult. We acknowledge that we could compare data from the two groups concerning the daily intake of dairy products and calcium and other characteristic in such a table, but the same data on the 315 included subjects that would appear in the new table already appear in both Table 1 and Table 2. Thus we decided not to include an additional table of this nature. For your reference, the tables below compare the included and excluded study populations.
Table A Characteristics of the study population (n=315) as compared with the non-participating or excluded subjects (n=687), OMCHS, Japan

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study population, Number (%)</th>
<th>Excluded subjects, Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 29</td>
<td>91 (28.9)</td>
<td>289 (42.1)</td>
</tr>
<tr>
<td>29-31</td>
<td>106 (33.7)</td>
<td>193 (28.1)</td>
</tr>
<tr>
<td>≥ 32</td>
<td>118 (37.5)</td>
<td>205 (29.8)</td>
</tr>
<tr>
<td>Gestational age at baseline (weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 15</td>
<td>111 (35.2)</td>
<td>246 (35.8)</td>
</tr>
<tr>
<td>15-20</td>
<td>94 (29.8)</td>
<td>235 (34.2)</td>
</tr>
<tr>
<td>≥ 21</td>
<td>110 (34.9)</td>
<td>206 (30.0)</td>
</tr>
<tr>
<td>Family income (yen/year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4,000,000</td>
<td>75 (23.8)</td>
<td>226 (32.9)</td>
</tr>
<tr>
<td>4,000,000-5,999,999</td>
<td>129 (41.0)</td>
<td>274 (39.9)</td>
</tr>
<tr>
<td>≥ 6,000,000</td>
<td>111 (35.2)</td>
<td>187 (27.2)</td>
</tr>
<tr>
<td>Maternal education (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 13</td>
<td>69 (21.9)</td>
<td>254 (37.0)</td>
</tr>
<tr>
<td>13-14</td>
<td>136 (43.2)</td>
<td>277 (40.3)</td>
</tr>
<tr>
<td>≥ 15</td>
<td>110 (34.9)</td>
<td>156 (22.7)</td>
</tr>
<tr>
<td>Paternal education (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 13</td>
<td>102 (32.4)</td>
<td>298 (43.4)</td>
</tr>
<tr>
<td>13-14</td>
<td>55 (17.5)</td>
<td>117 (68.0)</td>
</tr>
<tr>
<td>≥ 15</td>
<td>158 (50.2)</td>
<td>272 (39.6)</td>
</tr>
<tr>
<td>Maternal smoking during pregnancy</td>
<td>43 (13.7)</td>
<td>141 (20.5)</td>
</tr>
</tbody>
</table>
Table B Distribution of daily intake of dairy products and calcium at baseline in 315 study subjects as compared with 687 non-participating or excluded subjects, OMCHS, Japan

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study population, Mean (SD)</th>
<th>Excluded subjects, Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (kJ)</td>
<td>7557.5±1768.4</td>
<td>7682.4±2052.8</td>
</tr>
<tr>
<td>Total dairy products (g)</td>
<td>179.4±113.0</td>
<td>164.2±124.8</td>
</tr>
<tr>
<td>Milk (g)</td>
<td>128.6±101.2</td>
<td>119.2±112.6</td>
</tr>
<tr>
<td>Yogurt (g)</td>
<td>45.0±38.6</td>
<td>39.6±46.8</td>
</tr>
<tr>
<td>Cheese (g)</td>
<td>5.8±8.9</td>
<td>5.4±7.9</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>556.1±168.3</td>
<td>529.5±174.7</td>
</tr>
</tbody>
</table>

Nutrient intake and food intake were adjusted for total energy intake using the residual method

2. Supplement use was uncommon (6% used calcium supplements at least once per week). For unclear reasons, the authors omitted data on this supplemental calcium intake from their analysis. While a focus on dietary intake is fine, it would be helpful for the authors to report association between total calcium intake (with supplement included) and childhood caries.

Response:
In addition to the fact that only a small number of subjects used supplements, there is also the problem that, unfortunately, reliable composition tables are lacking for dietary supplements in Japan. Therefore, we were not able to analyze the association between total calcium intake including that from supplements and dental caries in children. We changed a sentence in the original manuscript from “Information on dietary supplements was not used because only a small number of participants (6.0%) used calcium supplements at least once per week.”(page 6, lines 17-19 in the original manuscript) to “Information on dietary supplements was not used due to the lack of a reliable composition table for dietary supplements in Japan. Also, only a small number of participants (6.0%) used calcium supplements at least once per week.”(page 6, lines 19-22 in the revised manuscript).

3. It would be helpful for readers to understand the contribution of each individual food to the total calcium intake of mothers.

Response:
We were not able to obtain data on the contribution of each individual food to the total calcium intake because the ad hoc computer algorithm for the DHQ has not been disclosed by the developer of the DHQ. According to estimates of daily intake which were obtained from the developer, the mean calcium intake from total dairy products, defined as milk, yogurt, cheese, and ice cream, accounted for $39.9 \pm 16.0\%$ (range: 0 to 70.0\%) of the total calcium intake in the study subjects. In our present study, on the other hand, total dairy products intake was defined as milk, yogurt and cheese intake. Intake of ice cream was not taken into consideration in our analyses. Thus the contribution of total dairy products to total calcium intake assumed in our study is likely to be lower than that assumed by the developer of the DHQ. We did not describe this circumstance in the text.

4. Moreover, what was the correlation between each of the major calcium-containing foods and calcium intake? (to help address co-linearity)

Response:
In the present study, Pearson’s correlation coefficients between maternal intake of milk, yogurt, and cheese and total calcium intake were 0.74 (95% CI: 0.68-0.78), 0.43 (95% CI: 0.33-0.52), and 0.38 (95% CI: 0.28-0.47), respectively. We added the sentence “Pearson’s correlation coefficients between maternal intake of milk, yogurt, and cheese and total calcium intake were 0.74 (95% CI: 0.68-0.78), 0.43 (95% CI: 0.33-0.52), and 0.38 (95% CI: 0.28-0.47), respectively.” to the Results section (page 9, lines 7-9 in the revised manuscript)

5. To address linear trends, the authors assigned median value for the population to each category and used these values as a continuous variable. Why not just use the actual value for this test of linear trend? What did that approach show?

Response:
When actual values were used in the test for linear trend, P values for trend of total dairy products, milk, yogurt, cheese, and calcium were 0.09, 0.54, 0.007, 0.03, and 0.14, respectively. In that case, the association between dairy products other than yogurt and the risk of dental caries was attenuated, as was that between calcium and the risk of dental caries. Therefore we decided to estimate the linear trend using the median value for each category. The same method has been used in a number of other nutritional epidemiological studies. We did not mention this issue in the text.
6. The authors state that after adjustment for calcium the association between total dairy products and yoghurt and childhood caries “disappeared” (Results and Discussion) – but the point estimates actually are quite similar: 0.50 vs 0.57 and 0.50 vs 0.58, respectively. While the P-value no longer is <0.05, I would not conclude from these findings that the association had “disappeared”; it’s more likely a question of co-linearity and statistical power.

Response:
To examine whether the association of maternal intake of total dairy products, yogurt, or cheese during pregnancy with the risk of dental caries in children could be attributed to calcium intake, we conducted an additional analysis in which we adjusted for maternal calcium intake during pregnancy. If maternal intake of calcium contributes to the associations between the intake of each dairy product and dental caries, the effect estimates are altered due to collinearity. This method is often used in epidemiological studies.

Although the differences between the point estimates made before and after adjustment were certainly small, the inverse associations between maternal intake of total dairy products and yogurt and the risk of dental caries were not statistically significant after further adjustment for maternal calcium intake during pregnancy, whereas before adjustment they were marginally statistically significant. We changed the term “disappeared” to “were attenuated” in the Results section and the Discussion section (page 10, lines 6 and 23 in the revised manuscript)

DISCRETIONARY REVISIONS

Why would cheese be protective and not milk? The authors provide an interesting discussion that highlights the possibility of a spurious association (eg, that maternal intake correlated with childhood intake and it’s the latter that matters). Is there evidence that this explanation is unique to cheese? Wouldn’t maternal milk intake and childhood milk intake also be correlated? It would be helpful if the authors could elaborate on this interesting explanation.

Response:
We have no immediate explanation for the potential mechanisms underlying our observed associations. As we had mentioned in the text, intake levels of foods and nutrients are likely to be correlated between mothers and children (page 11, lines 16-17 in the revised manuscript). In the present study, information on the dietary habits of the children over the month prior to the fifth survey was available. The Pearson’s
correlation coefficients between maternal intake of energy-adjusted milk, yogurt, and cheese during pregnancy and children’s intake frequency of milk, yogurt, and cheese at fifth survey were 0.22 (95% CI: 0.11-0.32), 0.16 (95% CI: 0.05-0.26), and 0.08 (95% CI: -0.03-0.19), respectively. Thus, in the present study, maternal intake of dairy products during pregnancy is likely to have little relation to the frequency of the children’s intake of the same dairy products. On the other hand, our previous cross-sectional study among Japanese children aged 3 years found an inverse association between intake of yogurt, but not cheese or milk, and the prevalence of dental caries in children. This is at variance with our present findings.

We added the following passage to the Discussion section and the reference cited in it to the reference list:

“In the present study, however, the Pearson’s correlation coefficients between maternal intake of energy-adjusted milk, yogurt, and cheese during pregnancy and children’s intake frequency of milk, yogurt, and cheese at the fifth survey were 0.22 (95% CI: 0.11-0.32), 0.16 (95% CI: 0.05-0.26), and 0.08 (95% CI: -0.03-0.19), respectively. Thus, maternal dairy products intake during pregnancy is likely to have little relation to the children’s frequency of dairy products intake in the present study. Our previous cross-sectional study among Japanese children aged 3 years found an inverse association between intake of yogurt, but not cheese or milk, and the prevalence of dental caries in children [23]. This is at variance with our present findings.” (page 11, line 20 – page 12, line 2 in the revised manuscript)