Dear Editor,

Thank you for giving us the opportunity to improve and resubmit our manuscript “Plasma phospholipid pentadecanoic acid, EPA and DHA, and the frequency of dairy and fish product intake in young children”. Please find enclosed the revised manuscript for further consideration.

The manuscript has been revised according to the comments raised by the reviewer to the best of our ability. Changes to the manuscript are underscored and highlighted in red. Please find a detailed reply to the reviewer comments attached with this revision.

We would like to thank the reviewer for the constructive and competent criticism, and we hope that our manuscript will be acceptable for publication in Food & Nutrition Research.

Response to the reviewers

Reviewer: 1

Comments to the Author

This is a interesting and useful work. But the authors fails to provide strong statistical justification of their conclusions based on available data. The data analysis presented in the paper is really novice and much more need to be done to clarify the hidden truth behind the data. Below are some such observations from the data reported in the paper which surely need to be addressed/clarified before the manuscript can be re-considered again for publication.

1. Since there is a lot of different between the means & SD with the Median & IQR for some variables (for example, Yoghurt, Cod liver oil etc), outlier analysis must be done before any kind of analysis with this data. Authors mentioned that the fact is due to non-normality of the data but no prove supporting this has been provided. Authors should provide normality test and outlier analysis (e.g., box plots etc) in order to confirm the actual reason for this discrepancy. If there is any outlier in any variable, those observations need to be removed from all subsequent analysis which might alter their conclusion (some potential outlier can be seen from Figure 2).

Reply:

We thank the reviewer for this important suggestion. We have now provided normality test and outlier analysis (please see Figure A1 and A2 attached to this response letter), which supports the use of spearman correlation analysis. To identify potential outliers we have, in addition to box plots of all variables, also studied residual- and scatter plots. We identified eight potential outliers and we have followed the reviewer’s suggestion to remove these outliers from all subsequent analysis. This resulted in small changes in some correlations but did not lead to any major changes that had implications for our conclusions. The outliers removed could be biologically plausible, but since the Spearman correlation coefficients did not change substantially, we have decided to present these data in line with the reviewer suggestions.

Due to space limitations we have decided not to include the box plots and normality test in the manuscript, but these can be included at the editor's discretion.

2. All the combined artificial variables considered in Table 3 & 4 are correlated among each other (which is not verified in the paper). So, the conclusion drawn from this correlation analysis are sometimes seems spurious. For example, in Table 3 , author showed three important variables (Cheese, Total fat diary & Total dairy) having high correlation with the Pentadecanoic acid, but it seems that actually there is only one important variable Cheese effecting the acid measures as the high correlation with the other two mainly comes from the fact that cheese is also included in there definition (and if subtract cheese from their definition the remaining part will have non-significant correlation). Hence the authors' conclusion in this regard is not fully justified and they should explain this issue more clearly with improved conclusion. Same comment appears for Table 4 with Total oily fish & Total fish.

Reply:

We thank the reviewer for this important criticism. The combined variables were a priori combinations of foods we wanted to investigate. As the reviewer noted, the different combined variables often contain the same underlying variables, and some of these (such as cheese) contribute more than others to the reported correlation, which was not apparent enough from our manuscript. We have now stated that cheese is the main contributor behind these correlations more clearly in the manuscript: "Of all dairy products cheese had the strongest correlation with the concentration of plasma pentadecanoic acid. The moderate correlation between the intake frequency of total fat dairy and pentadecanoic acid was mainly explained by the contribution from cheese." (page 14)

We have also discussed the variable total oily fish in regard to the other comments (see reply below).

3. There is no proper explanation or conclusion from Figure 2 in the paper. Apparently just looking at the figure, there are several anomaly in the relevant analysis. As mentioned previously, there is a clear outlier in data in the group of "No cod liver oil" and after removing that one the correlation value for that group will change a lot!

Reply:

We thank the reviewer for pointing this out. We have now followed the suggestion from the reviewer and removed this observation from our analyses, together with seven other observations which were considered as outliers. We have now described the new results in an updated version of figure 2. The Spearman correlation for the subpopulation that did not use cod liver oil did not change substantially, from r=0.23 before to r=0.22 after removal of outliers. However, we noticed that the more sensitive pearson correlation coefficient changed for the subpopulations. Among those who did not use cod liver oil the pearson correlation coefficient changed from r=0.13 to r=0.23. We have now improved the discussion of the results in the manuscript (see reply below). We would also like to apologize for a mistake in the previous version of figure 2; the presented correlation coefficient for the subpopulation of those who did not use cod liver oil reported pearson correlation (r=0.13) and not spearman correlation (r=0.23) as intended. This has now been corrected in the revised version of the manuscript.

4. Further, it is not justified statistically to combine two such group having so different values of correlation and it is also not clear from the paper what the author want to conclude from the combined measures given in the figure. Correct analysis of the data (after suitable outlier analysis for both the groups) and proper conclusion from that analysis need to be provided.

Reply:

We understand that our intention behind the figure was not clear from what was described in the paper, and we thank the reviewer for pointing that out to us. We have not combined these two groups in one analysis, but we have presented the results from the correlation analysis of total oily fish and DHA for the whole sample, and from the correlation analyses of total oily fish and DHA for the subpopulations of those who used cod liver oil and those who did not, while showing the distribution. The analyses are described in the data analysis section.

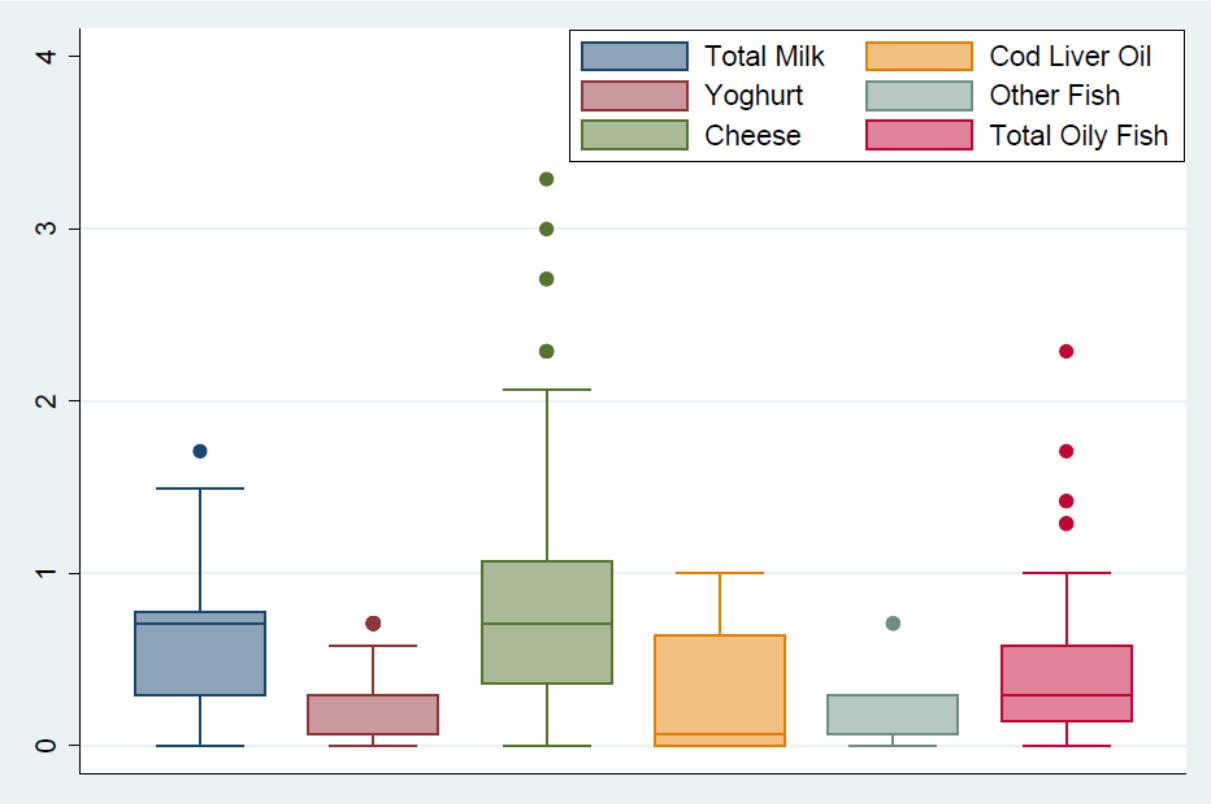
We have now made this clearer by describing the content of figure 2 in a footnote: “Distribution of the intake frequency of total oily fish estimated with a food frequency questionnaire (FFQ) and the plasma phospholipid DHA (μg/mL), and Spearman’s correlation coefficients for the whole sample (n=102), and subpopulations of those who used cod liver oil (n=55) and those who did not (n=47). In total 8 observations were considered outliers and are not shown nor used in the correlation analyses.”

It is plausible to think that those who consumed oily fish also consumed cod liver oil as part of a health related behavior, and we wanted to investigate if the correlation between the intake frequency of total oily fish and DHA was different between the subpopulations who consumed cod liver oil and those who did not. This could be of interest to the readers and our intention was to show the distribution and correlations in one figure.

We have now discussed this in the manuscript: “There was a lower correlation between total oily fish and DHA for the subpopulation of those who did not use cod liver oil than for those who did, indicating that the correlation between total oily fish and DHA could in part be explained by the use of cod liver oil. However, these data has to be interpreted with caution as the number of participants was lower in the subpopulation analyses (n=47 and n=55, respectively), and it has to be taken in to consideration that a majority of the children who consumed oily fish most frequently also consumed cod liver oil.” (page 14)

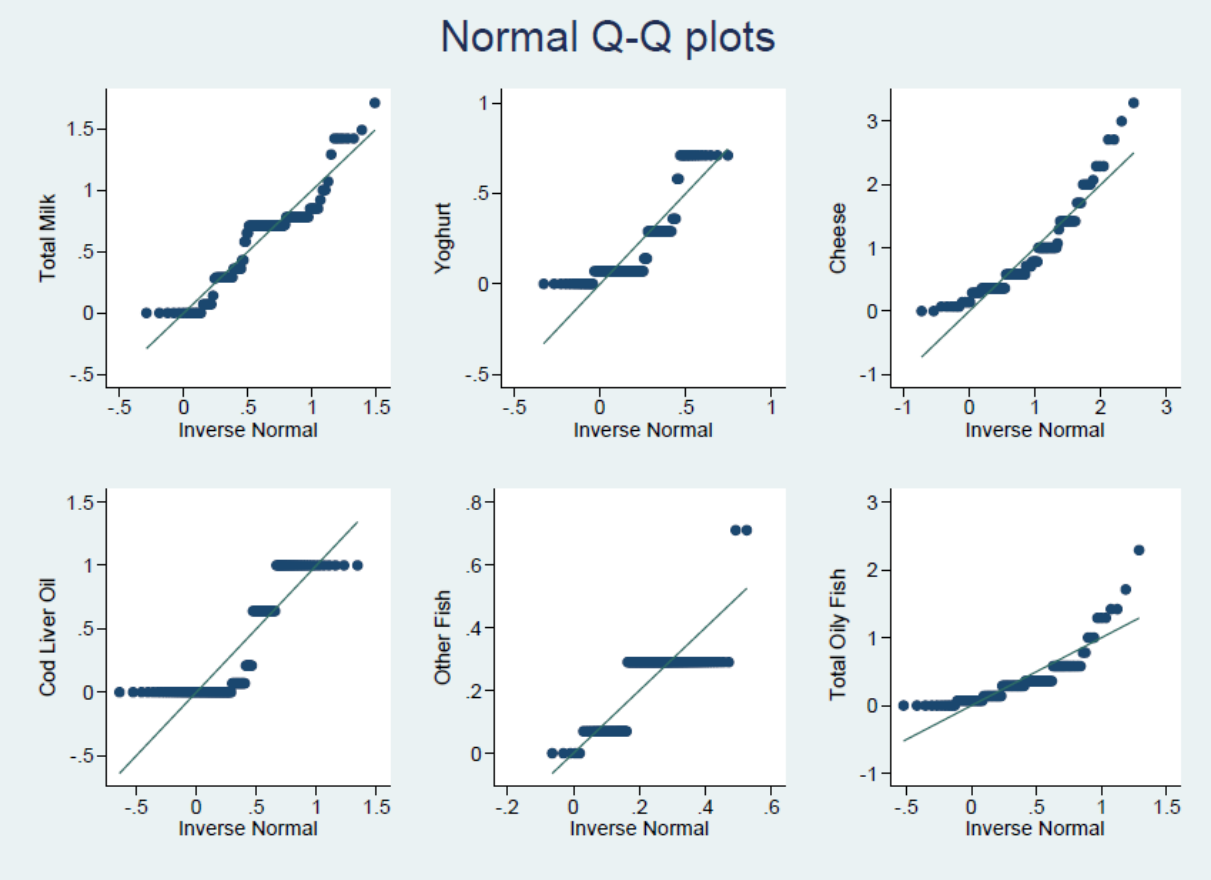
Attachments

Figure A1. Box plots showing the distribution of the intake frequency of selected food items



The horizontal line in the box represents the median. Upper limit of the box represents Q3. Lower limit of the box represents Q1. Upper whisker represents the maximum observation within the upper inner fence (Q3+1.5IQR). Lower whisker represents the minimum observation within the lower inner fence (Q1-1.5IQR). The dots represent outside values. Shapiro-Wilk W test for normal data showed $p<0.001$ for all variables

Figure A2. Normal Q-Q plots of the intake frequency of selected food items



Shapiro-Wilk W test for normal data showed p<0.001 for all variables