Author's response to reviews

**Title:** Increased dietary alpha-linolenic acid has sex-specific effects upon eicosapentaenoic acid status in humans: re-examination of data from a randomised, placebo-controlled, parallel study

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**Author's response to reviews:** see over
Response to reviewer comments:

Reviewer 1:

Discretionary Revisions:
There is no information of the method used for the plasma PC fatty acid composition measurement. This could be mentioned very briefly.

**Further details of the method have now been included.**

Page 4 : "Correlations presented are Pearson's R-values." No correlations were presented.

**This line has been removed.**

Page 10 Table 1: The changes in fatty acids could be presented in this table and the baseline levels of omega-3 fatty acids in table 3.

**Baseline levels of omega-3 fatty acids have now been included (Table 1).**

Page 12 Table 3: Label this as a Table 1 and present it in the first chapter of the results as the characteristics.

**Table 3 has now been moved to become Table 1.**

Reviewer 2:

1. This is a very clear and concise manuscript and I have no major compulsory revisions.

Minor Essential Revisions
2. Abstract (Pg. 2) Line 4..."As far as we know there are currently no studies THAT have specifically..." (the word should be "that" and not "which" for proper grammar).

**Amended as described.**

3. Same comment as #2 above applies to Pg. 3 1st line of paragraph 2.

**Amended as described.**

4. Use either "sex x diet" or "sex * diet" (i.e. please be consistent in the wording of this)

**Manuscript was checked to ensure sex*diet used throughout.**

Discretionary Revisions

5. Pg 4 (Methods): It would be very helpful if the authors could provide the levels of ALA in the specially formulated margarines within the text of the Methods.

**A line describing the ALA content of the margarines used has been added to the methods.**
6. The authors state that "increasing BMI is typically indicative of increasing body fatness.". The lack of other measures, such as waist circumference or other body composition measures should be recognized as a limitation of the current work when exploring the relationship between adiposity and sex differences in fatty acid status. This section (Pg. 6) remains quite speculative given the data available.

A line has been added to indicate that “Further studies incorporating additional measures indicative of body composition and adiposity, such as waist circumference, will be required to further investigate these hypotheses.”

Reviewer 3:

Overall a very useful paper which attempts to better understand gender effects of LC n-3 synthesis from ALA. The work is based on a re-analysis of data from an earlier study but certainly deserves to be published. The paper is short and would benefit from some editing before publication. Suggestions for this are listed below:

Minor Essential Revisions
Title (and elsewhere)
1. Is this strictly a placebo-controlled study? The control was the low dose of ALA which is not a treatment-inactive substance.

Yes, the original study was described as placebo-controlled as all participants were provided with spreads and capsules to consume during the study - the ‘control’ margarine was a typical n-6 PUFA rich margarine based on sunflower and safflower oils.

Abstract
2. Lines 12, 13 include the length of the intervention period.

The six month intervention period has now been stated in the abstract.

Findings
Background
3. Line 6, it would be helpful to indicate overall how much greater was the capacity in females than in males in stable isotope studies.

Values have now been included in the text.

4. Line 8, confirm if females also had higher circulating levels of EPA. This is relevant to the outcome of the present study.

A line has been added to describe sex differences in EPA and DPA status described in this reference.

Methods

5. Line 10 (p4), whilst it is appreciated that this will be in the original paper on the study, it would be helpful to the reader if the numbers and ages of males and females in each of the two groups was given.
The number of participants in the original study and their ages, and the proportion of M/F in the control and high ALA diet have now been stated in the methods.

6. Line 14, whilst it is true that the main effect being studied was the diet x sex interaction, was the effect of diet not also included in the ANOVA? It would still be useful information.

   Individual p values for the effect of diet and sex were generated by the model, and have now been included in table 2. Text in the results section has now been added to indicate that significant effects of diet were observed for ALA and EPA content.

7. Line 16/17, it is implied but confirm that the independent variable was change in plasma PC EPA concentration from baseline to finish.

   Text has been added to clarify that data were expressed as change from baseline following the six month dietary period.

Results
8. P5, line 6, the term 'dietary intervention group' has previously been called 'diet'. Best to be consistent.

   Amended to ‘diet’ throughout as suggested.

Discussion and conclusions
9. P6, lines 3-4, it is mentioned that data on menopausal status were not available but since the mean age of the females was 53.5 +/-12.0 years is it not likely that a reasonable number would be within or post menopausal? If that is a reasonable assumption some mention of it would helpful in relation to the key finding of a sex effect.

   Additional text has been added to indicate the number of women in this dataset aged <45yr, and to propose that further studies will be required to investigate the potential role of menopausal status.

10. The Discussion does not consider the lack of a sex effect on DHA (or DPA) despite the Introduction highlighting increased circulating DHA in females seen in stable isotope studies. Some discussion of the possible reasons for this would be helpful together with the consequences if there are specific needs for DHA e.g. during pregnancy. This is also worth a mention in the concluding paragraph on p 7.

   The discussion now states that there are no significant differences in the DPA or DHA response to treatment, nor when males and females are compared at baseline. A line has also been added to the concluding paragraph.

11. Table 2. 'Intervention group' should be 'Diet' for consistency

   Amended to ‘diet’ as suggested.

12. Table 3. The title should confirm that the data relate to baseline (if true).
There should also be confirmation re the +/- values after the means, are they SDs?

**Line added to indicate that data are mean +/- SD.**

Discretionary Revisions
1. Results, Line 2, 'higher increase' is probably better as 'greater increase'

   **Amended as suggested.**

2. Table 1. This also shows a diet x sex effect for 16:0 but is not mentioned. Is this worthy of a mention?

   **The sex*diet interactions observed for 16:0 and 20:1n-9 have now also been called out in the text.**