Author’s response to reviews

Title: A pilot randomized controlled trial to promote healthful fish consumption during pregnancy: The Food for Thought Study

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Author’s response to reviews: see over
Reviewer 1

*Abstract:
1. Please add information on the setting (country) and time-frame (date study was conducted) to the methods.
Done

*Introduction:
2. The authors indicate that “Fewer than half of pregnant women in the US eat the amount of n-3 PUFA recommended for optimal maternal and child health.” Please state what this recommended intake is.
We have added this information (page 3).

*Methods:
3. When was this study conducted? Please provide dates for enrollment and follow-up.
Done

4. In the text and figure, please provide the number excluded for the 1-2 most common reasons, e.g., fish consumption >=2 times/month; gestational age.
We have now added this information to the methods section (page 5), but left the figure as-is (with the total number ineligible, not broken out by reason) as it appeared too cluttered when we included this information; however if the editors wish us to also include it we are happy to reconsider.

5. Where were the baseline and final follow-up visits conducted? Assuming these were in-person interviews, were the interviews conducted at a hospital or academic site or in the participants’ homes?
We have now added this information (page 5).

6. Biosample assay: For all assays, please provide information on the lower limit of detection and a measure of assay variability/sensitivity.
We have now added this information (page 9).

7. Please provide in an appendix the list of 29 recommended low-mercury fish and list of fish to avoid due to mercury content.
Good idea; we now include this information in a Box; we are happy to include with the published article or as an online supplement, upon the advice of the editors.

8. Analysis: Did the authors check the distributions of variables for normality? Based on the results, I wonder if medians or geometric means (using log-transformed data) may be a more appropriate method of summarizing some of these data.
We now present baseline data as median (IQR) for continuous variables (Table 1). Because change variables were more normally distributed, we keep them as means (SD).

*Results
9. Based on the mean, SD and ranges provided in the text and Table 1, the dietary variables appear to have right-skewed distributions, so the median and inter-quartile range may provide a better measure of central tendency (or geometric means).
Done, Table 1.
10. I am not sure how a p-value of 0.05 was obtained for the comparison of preterm birth between control and intervention groups. Please provide denominators, eg shouldn’t this be: 2/20 (10%) vs. 0/35? The analysis section says chi-square tests were used to compare dichotomous variables, but it is my understanding that an assumption of the chi-square test is that cell sizes should be above 5. The Fisher’s exact test for this comparison yields a p-value of 0.13 (not adjusted for multiple comparisons). On the advice of the reviewer, we now present the Fisher’s exact p value (0.12).

11. Despite a significant increase in dietary DHA in the 2 advice groups relative to the control group, plasma DHA did not change. How sensitive is the assay to detect small short-term changes on this measure? We now include in the limitations that 12 week follow-up may not have been long enough to allow detection of changes in biomarkers. We also cite literature that changes in plasma DHA or blood mercury after dietary interventions can be detected within a few weeks, at least with more dramatic dietary interventions (page 16).

12. Similarly, discussing the sensitivity (and lower limit of detection) of the mercury assays would be helpful in interpreting the results of this measure. Please see response to #6 above.

13. The standard deviations of many measures (dietary and blood in Tables 1 and 2) are quite large suggesting there is considerable variability associated with these measures, which makes it difficult to see differences without a larger sample. The authors need to provide more information (about the assay and the distributions of the dietary and serum variables) in order to increase confidence in their conclusion that no increase in mercury (dietary or serum) resulted from increased fish intake (or alternatively indicate that the study was underpowered to determine this). We now present the medians and IQR’s for the baseline measures. The distributions of the primary outcomes (change from baseline to follow-up) were actually quite normal (see response to reviewer 3 below).

14. The sample is too small to justify the statement “we found an apparent decrease in preterm birth in the intervention vs. control women.” As well, I think the authors should remove the last sentence of the first paragraph on page 15 (the lower rate of preterm birth among intervention women argues against...). We agree that this pilot study was underpowered for clinical outcomes, but since our (nonsignificant) finding suggesting a difference in preterm birth is supported by a more extensive literature on gestation length, we have opted to keep this mention in (page 14). However, we removed the suggested sentence on page 15.

15. The authors state that the results may not be generalizable to women outside the Boston MA area. Why not? Please be more specific. We now clarify that “results may not be generalizable to women living elsewhere with different access to fish.” (page 15).

16. Page 16: Please elaborate on the statement that methylmercury may confound the association between fish consumption and “health effects”. Which health effects in particular do the authors think that methyl mercury might affect? Eg, preterm birth? Depression? Inflammatory conditions? What evidence is there that methyl mercury affects these conditions? We now specify that methylmercury may confound benefits of fish intake for offspring neurodevelopment and adult cardiovascular disease (page 17).
*References:*
The links in Reference 31 and 34 no longer work and should be updated (I did not check links in other reference, but they should be verified as well).
We have updated all links in the references.

**Reviewer 2**

1. The introduction would be more balanced if the authors also would cite the studies implying benefits of maternal fish consumption on fetal growth and include references to lean fish also. 
   *Thank you for this suggestion, we now include (page 4) the statement that “consumption of lean fish during pregnancy may provide as much if not greater benefit than fatty fish for perinatal outcomes such as fetal growth [20-22].”*

2. Was the follow-up period of 12 weeks sufficiently long that it could be expected to find a difference in hair –Hg or should this mentioned/discussed among limitations?
   We now include this limitation (page 16).

**Reviewer 3**

1. I assumed that whole blood mercury means total mercury (organic and inorganic) and the unit is usually ug/L in many representative publication including NHANES and EPA publication. The authors already mentioned EPA benchmark dose using ug/L unit. But the authors used ng/g instead of ug/L. Please mention the particular reason to use ng/g instead of ug/L which is identical both unit. Otherwise, please use ug/L.
   We now report hair Hg as mcg/g and blood hg as mcg/L.

2. Table 1 describes baseline characteristics of 55 women and describes the statistical differences among three arms. Authors explained no differences among three arms in result section, but no indication of statistical significance. In some cases, readers do not read the result section and only look at the table. So please add statistical test results in the table 1.
   *In a randomized trial, by design any difference between arms at baseline is by chance – therefore, there isn’t really any meaning to a p value in this circumstance. While we could certainly calculate p values, we did not include them because we feel that it is not appropriate. For the reviewer’s information, all p values were in fact rather large (smallest p values = 0.10 for pre-pregnancy BMI and 0.16 for DHA from seafood). Furthermore, although our primary analysis was unadjusted, further adjustment for baseline characteristics made no material differences in our estimates.*

   Table 1 listed arithmetic mean with standard deviation for whole blood mercury and did not list the value of hair mercury level.
   *In Table 1, we describe measures collected at the baseline visit. Since we did not collect hair at the baseline visit, we did not include it here.*

   The SD of blood mercury is large and skewed, so it is recommended to present the data with geometric means with standard error for blood mercury and hair mercury level instead of AM with SD like the biomonitoring data presentation of NHANES.
   We now present data in Table 1 as median (IQR).
The authors also listed whole blood mercury \( \geq 3.5 \) ng/g(%). Please give explanation why you choose this level in the footnote.

Done.

3. Table 2 presented the change from baseline to follow-up by study arm. Authors applied chi square test separately advice and advice+GC with control group and concluded increased consumption of fish and DHA but not mercury by an educational intervention. The data can be analyzed using one way ANOVAs with individual comparison of tukey or other method. The data shows significant trend according to different arms. Please add trend test results if possible. I think one way ANOVA test is more appropriate for table 2.

We apologize for the lack of clarity, we did perform ANOVA tests for all the analyses of continuous outcomes included in Table 2. We used chi square (now Fisher’s exact) analysis for the dichotomous clinical outcomes.

Another suggestion: I found that the SDs are very large which indicates non normal distributions, and the data should be analyzed using Non parametric data analysis such as Kruskal-Wallis analysis. If the analyses results are same between regular ANOVA and non parametric statistical analysis, I recommend the latter method.

The distributions of the continuous outcomes (change from baseline to followup) were actually quite normally distributed, despite the relatively large SD’s (see examples below for change in total fish intake, and change in DHA from fish). Therefore, we have chosen to keep the presentation of outcomes in Table 2 as mean (SD) and the analyses as ANOVA.