Author's response to reviews

Title: A randomized longitudinal dietary intervention study during pregnancy: effects on fish intake, phospholipids, and body composition

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Version: 2 Date: 15 September 2014

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

Thank you for the reviews of our manuscript “A randomized longitudinal dietary intervention study during pregnancy: effects on fish intake, phospholipids, and body composition” (1129583922129918). We are grateful to the reviewers for their comments, which have helped us improve the presentation of our findings. We have rewritten the manuscript according to the recommendations of the reviewers and addressed their specific comments in the response to reviewers below. Some additional changes are described after the response to Reviewer 3. The entire manuscript has been reviewed by a native speaker of English with professional experience in editing scientific manuscripts for publication. These improvements have of course not changed the content of the manuscript.

The revised manuscript has been uploaded on your website.

All co-authors have read and approved the final version of the manuscript.

We hope our manuscript is now suitable for publication in the Nutrition Journal. Thank you for your consideration of our work.

Best regards,

Agneta Holmäng, MD, PhD

Marja Bosaeus, PhD student
Responses to Editor and Reviewers

Editor
Power calculation: accomplished (see Statistics).
Abstract structure: now more clearly structured.

Reviewer 1
The authors are very grateful for your extensive review, and our responses to your comments are listed below. Addressing your comments has greatly improved the manuscript.

STUDY AIMS
1. Although the title is really clear, the actual objective of this study is not. In fact, in the abstract the authors declare they want to investigate the effects of fish and meat intake on gestational weight gain, body composition, and serum phospholipid fatty acids in normal weight women during pregnancy, and only afterwards they declare they want to analyze the effect of a longitudinal dietary intervention. When reading the title and the background, where the objectives are declared, these aims are inverted. They need to clearly specify what is the main aim (it sounds like it is the assessment of the possible effect of the intervention) and what are the secondary ones, and give more emphasis on the main aim rather than on the secondary ones.

The aims have been revised in the abstract and in the background section. Specifically, we now state in the Abstract: “We aimed to determine whether a longitudinal dietary intervention during pregnancy could increase fish intake, affect serum phospholipid fatty acids, gestational weight gain, and body composition changes during pregnancy in women of normal weight. A second aim was to study possible effects in early pregnancy of fish intake and meat intake, respectively, on serum phospholipid fatty acids, gestational weight gain, and body composition changes during pregnancy.”

ABSTRACT
2. The p-values need to be reported precisely, not just indicating they are <0.05 (this applies to the whole text, not only to the abstract).

The p values are now reported as requested.

3. The abstract should be revised in order to describe what actually happened during the study; for example, it is said that a subgroup of women was studied during the whole pregnancy, while what actually happened is that a group of women was enrolled but some of them dropped out, thus the analysis on the whole pregnancy was only possible for a subgroup.

The subgroup is based on women who had no missing data. Thus, none of the women who were excluded from the subgroups analysis were drop-outs. However, some had missed a study visit (e.g., because of illness).

BACKGROUND
4. 3rd paragraph: please better explain which are the main PUFAs (EPA, DHA) and use the acronyms after their first explosion.

Acronyms are used. Three mistakes were found: ARA was not written as an acronym in a section of the Methods (Analyses of long-chain polyunsaturated fatty acids in serum phospholipids). Long-chain was not written as an acronym in the Background. Fat mass
was not written as an acronym in the Discussion (Gestational weight gain and body composition changes). We corrected these errors. EPA and DHA are also described.

5. 3rd paragraph: this paragraph mixes up information on both fish and meat and is not very fluid (for example, you wrote “Another important protein contributor in the diet is meat” but no other sources of protein were mentioned before.

The sentence with “Another important protein contributor in the diet is meat” has been revised to: “Meat is an important contributor of protein in the diet”.

At times, it seems you have just put together different sentences/concepts taken from your references without linking them together. Please try to keep the different components of your reasoning more connected.

Meat is already discussed in the end of this paragraph.

METHODS

6. The methods section includes information that should go into the results section. Methods should describe the study protocol, and not include what happened after the study was carried on. Thus, I suggest that the authors revise this section in order to clean it and leave only the actual methods. Moreover, this section should be reorganized and structured in order to neatly describe 1) what kind of study is this (interventional, randomized, controlled); 2) who are the patients (how were they recruited; inclusion and exclusion criteria); 3) randomization and how it was made; 4) description of the intervention(s), 5) how was the follow-up conducted (were visits usual controls women do during pregnancy or were they performed due to their participation in the study? ...and so on, using a logical order that allows the reader to understand how the study was conducted step by step. A figure could be provided to better outline the protocol.

Thank you for these suggestions. We have included a figure that shows the design of the study (Figure 1). The Methods section “Study design and participants” has been structured as suggested. Thus, previous parts of this section have been moved to Results, under the subheading “Study population”. The previous description of obese women who were enrolled in the PONCH study has been deleted from this manuscript (as they are not analyzed in this paper).

7. A definition is needed for “normal weight” and “obese”: were they classified according to the WHO definition, taking into account their BMI? What about the overweight women? Were they excluded? If so, as it seems, it needs to be specified and the reasons for this exclusion should be provided.

Normal weight BMI is now defined in the Methods (see Study design and participants). The description of the obese participants has been removed, since these women are not included in this manuscript. (PONCH has an obese control and intervention group, but these are not a part of the present analyses.)

8. BMI is self-reported (was it really a self-reported BMI or did these women just self-report their height and weight? Brackets explaining this were inserted) and this could induce a misclassification if the authors are not really sure about the baseline BMI. However, in other parts of the manuscript the authors describe how they calculated BMI, so it’s not clear which BMI was self-reported and how/when it was used.

This is now explained in greater detail. In the Methods, under “Study design and participants”, we inserted the following clarification: “Self-reported BMI was only used for inclusion”.

9. Was also diabetes self-reported? All the exclusion criteria should be explained.

Yes, the diabetes was self-reported. We have clarified this point in the revised manuscript in the Methods, under “Study design and participants”.

10. The authors wrote that women were recruited through maternity care centers but it is not clear how (advertisements? Health care personnel inviting them to participate?) and they do not specify if all Swedish maternity care centers collaborated recruiting patients for this study (it seems not, since the women all lived in a certain area).

In the Methods (Study design and participants), we inserted the following clarifications: “oral and written information given at” and “in Gothenburg (Sweden)”.

11. The authors wrote that all the women lived in a specific Swedish area but it is not clear if this was a specific choice or just a result (in this case it should go into the results section).

In the sentence about recruitment at maternity care centers (Methods, under “Study design and participants”) we inserted “in Gothenburg (Sweden)”. Thus, women were recruited in the Gothenburg area.

12. Please better explain how the randomization was made. Since the program was based on age, BMI and parity, it is necessary to understand the objective of this randomization program, i.e. if the program aimed to balance the groups according to these variables, and how exactly (obese vs normal weight? How was parity considered? Were women divided in age classes?).

In the sentence that describes the randomization (Methods, under “Study design and participants”), we replaced “based on” with “subjects were matched for”.

13. Criteria for excluding women from the analysis should be described in this section, but the number of women actually excluded should be only specified in the results section. Methods should be part of the study protocol, and not include what happened after the study was carried on. For the same reason, drop outs should be described in the results section, as well.

Now accomplished.

14. When was the first visit (enrolment) performed? And when were the others?

Under “Study design and participants” in the Methods, we added the following clarification: “Women were included and the first study visit took place during the first trimester (pregnancy weeks 8–12). Follow-ups were done in the second trimester (pregnancy weeks 24–26) and third trimester (pregnancy weeks 35–37) (Figure 1).” We hope the new Figure 1 will help make this clearer.

15. Was a sample size calculation performed? The authors do not declare how the number of participants was decided.

A power calculation has been inserted.

16. Was the subgroup analysis decided before the study was conducted?

No.

17. The authors wrote that women compiled a food frequency questionnaire to ascertain their weekly intake of fish and meat; it would be useful to know which were the items on this questionnaire; moreover, was this tool validated, as well?

In the “Questionnaires and dietary assessment” section of the Methods, we now state that the questionnaire was “developed at this department”. This questionnaire has not been
validated. Participants were asked to state the number of hot meals of fish/shellfish and meat per week.

18. The authors wrote “Participants received dietary counseling on the day of their visit to Sahlgrenska University Hospital for the other measurements in the PONCH study.” But this hospital was never mentioned before.

The hospital is now mentioned in Methods (see “Study design and participants”).

19. Counseling: women were advised “to lower sugar intake to <10 E%”; was this the actual advice or were the women invited not to consume more than a certain quantity of sugar using a more comprehensible unit of measure (such as number of teaspoons for example)? Similarly, were the women invited to eat 500g fruit/vegetables or to take a number of servings equal to…? Moreover, how were these women helped realizing what could they eat in order to increase their calories assumption of 350 or 500 kcal? Were they invited to eat certain types of food to increase calories?

The dietary counseling is now explained in greater detail (see Methods, under “Dietary intervention”). Specifically, we inserted the sentence “Additionally, women were advised to generally lower sugar intake in order to reach <10 E%.” We also revised the sentence about vegetables and energy intake, which now states: “Furthermore, women were advised to eat 500 g vegetables and fruits per day, and finally to increase daily energy intake ….” We also inserted the sentence: Advice on suitable amounts and choices of vegetables and fruits and appropriate snacks was given.”

20. Women were contacted on the phone “three times between the first and the second trimester and twice between the second and third trimester”; actually, it is not clear; it should be specified how many times they were contacted in the first/second/third trimesters and especially the frequency of these phone calls (were they made monthly?).

To clarify this issue, we revised the sentence as follows: “three times between the study visits in the first and the second trimesters and twice between the study visits in the second and third trimesters.”

21. Statistical analysis: parametric and nonparametric tests were used, and mean/standard deviation or median/quartiles were reported for different variables; moreover, both Pearson’s and Spearman’s correlation coefficients were calculated. However the authors did not specify if a check for normality was performed in order to decide how to describe and/or analyze data.

The decision to use nonparametric tests for the subgroup analysis was mainly based on the small sample size. However, some variables were also checked for normality in histograms and were not normally distributed in the subgroup.

RESULTS

22. The results section should start with a description of the number of patients: how many were enrolled, the randomization process, how many took the first visit in each arm, how many were followed up at the second visit in each arm… (description of the drop outs). Moreover, the results section should not be a rewriting of the tables.

The Results now begins with a new section (“Study population”), which describes the inclusion, randomization, participation in study visits, and number of drop-outs. A figure (Figure 1) has been included to illustrate the study design.
23. TABLE 1 should be rewritten:
   a. Report all P-values (“ns” needs to be avoided)
      Accomplished.
   b. Include the number of women described (“n”)
      Already described.
   c. Specify whether the BMI was the one calculated by the researchers or self-reported
      Now explained in the Methods section that self-reported BMI was only used for inclusion.
   d. Parity should not be used as a continuous variable (a mean of 0 and a SD of 0.1
      (children!) do not convey much information), since many of these women were
      primiparous; frequency of women that are primiparous and those that already have 1 or 2
      or… children (the authors should choose according to the distribution, it should be
      probably convenient to make classes) seem more appropriate.
      Parity is already described with median and interquartile range (0, 1) and not as 0.1.
   e. Could education be better categorized (does “more than 15 years” mean they have a
      university degree? Yes.), also in order to understand the distribution of education?
      This present description of education should be enough for this manuscript; education is
      not a major factor, rather a descriptive data in this paper.
      Maybe this can be specified in the text? Moreover, a p-value for this comparison is not
      provided.
      Differences between groups were not tested.

24. In the “Results at baseline” section the authors reported that none of these women smoked
    during pregnancy, thus it is not clear whether the information refer to the first period of
    the pregnancy or to the whole pregnancy.
    It refers to baseline. “during pregnancy” was deleted and replaced with “during the first
    trimester”.
    The subgroup should be described separately.
    The subgroup of the women is included in the pooled baseline population. Thus none of
    them smoked in the first trimester.

25. A table showing the (none) differences between the women who completed the study
    and those who were lost at follow up should be provided, in order to evaluate a possible
    attrition bias.
    It is not feasible to show so much data.

26. Comparisons between supplement users and nonusers should be avoided, since numbers
    are too low.
    A new sentence has been inserted in Results (see “Early pregnancy”): ”In the baseline
    population, three women used supplements containing fatty acids” (please see below).

27. Supplements with fatty acids paragraph: Leave out the description of statistical
    differences. Comparisons are not appropriate.
    Statistical comparisons have been removed throughout the manuscript (in Table 7 and in
    the Results sections “Early pregnancy” and “Supplements with fatty acids”. Thus, the
    section “Supplements with fatty acids” has been shortened.
28. TABLE 2: n (number of women) needs to be specified in the column header or, if the variables have different n, just after the name of the variable (e.g. “Parity (n=x)”; moreover, the same comments as for table 1 apply.

N is sometimes described with ranges in both Table 1 and Table 2. We believe this approach helps keep the tables simpler and more reader friendly.

29. TABLE 3: n (number of women) needs to be specified in the column header or, if the variables have different n, just after the name of the variable (e.g. “Parity (n=x)”; I would suggest the author to “turn” the table, having the values of meat/fish/energy intake on the lines and the three trimesters as the column headers; P-values need to be reported.

P values are now reported. We kept the previous lay-out (please see above).

30. TABLE 4: Same comments as above.

See above.

31. TABLE 5: Please specify the “n” and report the p-values for each correlation coefficient you calculated.

P values are now inserted in Table 5.

32. TABLE 6: N and p-values.

See above.

DISCUSSION

33. The discussion section is too long and reports too many details of other studies. References should be used only to compare and discuss study data. Authors should avoid to simply summarize other literature evidences if not necessary.

We have shortened (and we hope have improved) the discussion as recommended.

34. “A high percentage of the women reported a satisfactory fish intake at baseline; higher than the 33 g/day among women aged 31-44 years in a Swedish national survey” Please estimate the daily intake of the women enrolled in this study in order to make this comparison clear.

In the discussion we now state that fish consumption was 384 g/week, or 55 g/day.

35. Please consider (and discuss) the possibility that these women, coming from the same geographic area, influenced each other; in fact, women assigned to the intervention group could be in touch with others assigned to the control group, thus exchanging the information received and diluting the effect of the intervention.

Gothenburg has a population of 533,000, so this is probably not a major source of error.

36. As for PUFAS, “Both correlated with fish intake in the first trimester but not later in the pregnancy, perhaps because fewer women participated in the second and the third trimester, but also because of possible report bias”. Actually, this difference is really difficult to explain, especially because it is clear in the first trimesters and disappears afterwards. However, what can be hardly said is that a correlation could have been missed due to low numbers (unless numbers are so low an analysis should be avoided since it doesn’t make sense). In fact, when numbers are low, correlations may become non-significant, but the correlation coefficients should not be so different.

True point. This sentence has been revised to: “Both correlated with fish intake in the first trimester and s-DHA correlated with fish intake in one of the groups in the second trimester”. There was a misprint of a positive significant correlation in one group for s-
DHA, second trimester, in Table 5. This has now been corrected; see a thorough explanation further down.

37. “Also, there was a nonsignificant trend toward increased s-EPA in the intervention group. In nonpregnant conditions, the fatty acids in blood are extensively used biomarkers for fatty acid intake [28], as these only to a limited extent are endogenously synthesized from #-linolenic acid [29]. Fat metabolism is altered during pregnancy; initially fat is stored in the fat depots, but later in pregnancy breakdown of fat tissue [30], leads to higher levels of free fatty acids in the blood [31]. Pregnancy itself affects the fatty acid profiles of the mother, owing to the natural fat deposition that occurs during this period [32, 33]. Plasma phospholipid concentrations increase during pregnancy [34], and there is an active transport of PUFAs, particularly DHA, across the placenta to the fetus [35]. Also, it might be possible that EPA and DHA are consumed during pregnancy for production of eicosanoid-derived mediators like prostaglandins. We have earlier observed such consumption during inflammatory states when prostaglandin production is needed [36, 37].” All this paragraph is not sufficiently put into context.

We have tried to put this information into a better context.

38. “Yet, the increase in fish intake could not be verified by positive correlations with the serum fatty acid levels, possibly because of uptake of the fetus or dilution in the increased blood volume. Therefore, new tools and biomarkers should be identified that could help support reported food intakes in pregnant women.” Actually, two of the reasons for the lack of correlation are reporting bias and the questionnaire on the intake of fish and meat, that doesn’t seem to be validated as the one used to estimate the energy intake.

Reporting bias is discussed in the previous section in the Discussion. A new sentence has been included in the last paragraph of the Discussion: “Also, errors may have been introduced by the use of a food frequency questionnaire that has not been validated and that uses standard portion sizes.”

39. “There is however no reason to expect greater over-reporting by controls than by women in the intervention group.”. Well, I think there is, since in the intervention group women received information about modifying their dietary habits, while those in the control group did not. Social desirability bias could be more present in the intervention group.

There seems to be a misunderstanding here; we suggest that there is no reason to expect greater over-reporting in the control group.

40. “Thus, intake of fatty or lean fish should not be a source of error for lack of correlation between s-DHA and s-EPA and reported fish intake or (although nonsignificant) differences in s-EPA between groups.”. Right, and recommendations should take into account this difference, not only thinking about a different content in PUFA but also because in fatty and lean fish the content in mercury and other pollutants (such as PCBs) is different. Therefore, when a counseling is programmed, specific information should be given to mothers-to-be on the type of fishes they should prefer when increasing their consumption.

Advice was given on types of fish, according to Nordic nutrition recommendations. Specifically we inserted the following clarification in the “Dietary intervention” section of the Methods: “and advice was also given on the types of fish to consume to avoid pollutants”.

41. “The correlation in early pregnancy between s-ARA in the analyses of the larger baseline group and the meat intake in the first trimester also validates the reported meat intake”.” There are also other dietary sources of ARA that we did not study, such as eggs” It should be discussed that meat and eggs are not the only sources of ARA, that also comes a lot
from oils/dressings, thus not only specific foods but also the way they are cooked is important.

We have already discussed other possible sources of ARA such as for example eggs, but more extensive explanations are beyond the scope of this manuscript.

42. “Additionally, the size of the study population was rather small, which may have reduced the power to find differences or correlations” “thus reducing the precalculated statistical strength of the study.” this is the first time power is mentioned… The authors don’t mention any difference to be found in the methods section, nor the rationale for a sample size calculation. So please report your considerations on sample size in the methods section in order to comment on this.

A power calculation has been inserted into the Methods under “Statistical analyses”.

43. “However, as fish and shellfish are important contributors of fatty acids, vitamin D, proteins, and minerals, it is advisable that women of child-bearing age receive proper guidance to choose fish from nonpolluted waters.” This is not just a matter of nonpolluted waters but also of type of fish, as I already mentioned above. …

In that sentence, we inserted “the right types of” between “choose” and “fish”.

44. Consider the possibility of nausea/vomiting modifying dietary intakes and biological samples concentrations in the first trimester; was this information collected? This could partially explain several differences.

No, data on this was not collected.

CONCLUSIONS

45. Please rewrite conclusions in order to provide the implications for practice and (eventually) for research. Please avoid just reporting the statistically significant results.

We elected to retain the conclusions, as they present both implications for practice (counseling increases fish intake) and correlations that could inspire for further research.

Minor Essential Revisions

1. Background, 2nd paragraph line 3: “nevertheless in women with normal weight” should be replaced by “in fact, even in women with normal weight”.

Accomplished.

2. Background, 2nd paragraph “On the other hand, low GWG in normal weight women increases the risk for babies that are small for gestational age [2] and for babies <3000 g [3] and is associated with shorter gestation [8]” should be replaced by “On the other hand, low GWG in normal weight women increases the risk of giving birth to babies that are small for gestational age [2] or <3000 g [3] and is associated with shorter gestation [8]”

Accomplished.

3. Background, 5th paragraph: “To achieve healthy weight gain” should be connected to the previous sentence, for example using a “nevertheless”, that conveys the right significance to the importance of studying the entire diet when compared to the analysis of supplements intake.

“Nevertheless” was inserted.

4. Methods; A reference where the methods of the PONCH study are deeply explained would be appreciated.

So far, these papers are not yet published.
5. Results. Early pregnancy paragraph: when describing mean and standard deviation, please declare the one in bracket is SD, otherwise it is not clear.

The use of mean (SD) is described in the Methods under “Statistical analyses”.

6. Data on fish/meat/energy intake and on serum PUFA concentration should be reported in a table, where this information could be more readable.

Do you mean for the “early pregnancy” analyses here? (Tables 3 and 4 report this information for the subgroup analyses.) The “early pregnancy” analyses were pooled in one section (except for their baseline descriptive data) with the aim of compiling these data to make it more reader friendly.

7. The number of fish servings per week should be added to a table.

Not feasible, too much data.

8. TABLE 7: A description of the frequency of users/nonusers of supplements (what kind of supplements?) should be provided in the descriptive tables; a description of the items requiring the information on the assumption of supplements should be provided, as well. However, these low numbers don’t allow for comparison (max 4 users! More, is this information reliable? What about the use of supplements in pregnant women in Sweden?

Statistical analyses have been deleted.

Reviewer 2
The authors are to be commended for their attention to fish intake consumption in pregnancy; undoubtedly a very important issue. I have some comments on the manuscript:

1. What was the sample size calculated?

Power has now been calculated.

2. In table 1 and 2, please, include the mean of gestational week of each group. Despite have followed a visit protocol women may have a small difference in gestational weeks; it is important to readers look at these numbers.

Not feasible.

3. Page 12. “Gestational weight gain and body composition”: Authors described a correlation of FM and FFM with fish and meat intake, but data were not shown. Please, show these values on the manuscript (if not in table, provide a description in the text). This description is especially important because authors highlight this as a main result on abstract, discussion and conclusion. It is also not clear if this result was significant or borderline: - Abstract states: “Intake of meat in early pregnancy may increase the gain in fat-free mass..” – Conclusion states: “Meat intake in the first trimester is positively associated with gain in FFM”.

The correlations were not significant in the subgroup analyses (see Results, under “Gestational weight gain and body composition during pregnancy”). However, meat intake in the pooled early pregnancy analyses correlated positively with FFM gain.

4). Regarding possibility of over estimation fish intake by participants, it is also important to highlight 2 issues:

4a. Did authors calculate the food composition of specific fish (specie) reported by women in the food questionnaire?

No, species were not analyzed.
4b. Considering that authors estimated intake of fish using a dietary questionnaire and applied a standardized portion size (150 grams) it may be overestimated the intake of fish. Thus, a concern regarding the dietary method applied in the study should be address by authors in the discussion section. A food questionnaire with a standardized portion of fish may be not the best approach for a clinical dietary investigation. A weighed food diary would possibly be the best method. 

  Accomplished in the last paragraph of the Discussion.

5. In tables, Please insert a column for P values and don’t summarize all significant P values as “<0.05” or” <0.01”.

  P values are now reported.

6. The significant result of baseline intake of meat and gestational weight gain could be better investigated by authors; maybe with a different statistical approach.

  Not feasible at present.

7. A different statistical approach, rather than correlation, may be better (as regression or dose-response). Did authors try to examine the frequency of consumption rather than estimate portion size and grams of intake?

  A professional statistician approved the use of grams instead of frequency. Regression was not used for analyses.

**Reviewer 3**

This article deals with an interesting issue with an intervention trial. However, there are several questions need to be addressed.

1. The sample size seems small. Is it power enough to test the difference?

   Yes, the power is sufficient. See the power calculation in Methods, under “Statistical analyses”.

2. It is better to show the exact P value for each index, not just “ns”.

   Accomplished.

3. In conclusion, the effect of increasing fish intake on serum n-3 LCPUFA level and body mass should be stated.

   No significant correlations with gestational weight gain were found in any trimester (see Results, under “Gestational weight gain and body composition during pregnancy”). Concerning the effects of fish intake on serum n-3 PUFA: In the last sentence of the conclusion, we stated that fish intake in early pregnancy is correlated with serum phospholipid EPA and DHA. The correlations disappeared in later pregnancy and this was thoroughly discussed in the Discussion “Serum phospholipid fatty acids”.

**Additional changes**

1. A thorough review of the numbers/results uncovered some mistakes in Table 5 which have been corrected (as indicated by highlighting in the table). The corrections included one significant correlation, between s-DHA and fish intake in the second trimester in the control group. This positive correlation is now mentioned in the Results, under “Fish and meat intake and serum phospholipid fatty acids”; “...and in the second trimester in the control group,” was inserted.
2. In the Discussion, the section “Serum phospholipid fatty acids” has been revised as follows. a. The second sentence: “but not later in the pregnancy” has been replaced with “and s-DHA correlated with fish intake in one of the groups in the second trimester”. b. “Also, there was a nonsignificant trend toward increased s-EPA in the intervention group” was deleted. c. The last sentence in the second paragraph: “or (although non-significant) differences in s-EPA between groups” was deleted. d. The fourth paragraph, third sentence was deleted (“Possibly we had too few subjects to find correlations between s-DHA and fish intake in the second and third trimester.”). e. The fourth paragraph, fourth sentence was changed to: “A larger study failed to find any correlation between s-DHA and fish intake in the third trimester”.

3. Two figures have been added. Figure 1 describes the study protocol and Figure 2 the recruitment, exclusions, drop-outs, and final analysis groups. Figure titles and legends are added after the reference list.

4. In the Methods, under “Statistical analyses”, we deleted the phrase “and who used supplements containing fatty acids”.

5. In the Results, the section” Supplements with fatty acids” has been revised and statistical analyses were removed as recommended.

6. The Results now begins with a new section (“Study population”), which describes the enrolment, exclusions, and drop-outs. Part of this section was moved from the Methods section “Study design and participants”.

7. In the Results section, under “Early pregnancy” we now state that correlations between fish intake and GWG, FFM gain, and s-ARA were nonsignificant and correlations between meat intake and s-DHA and s-EPA were nonsignificant.

8. In the title, “serum fatty acids” has been replaced with “phospholipids”.

9. A new founding source has been added in Acknowledgments: Olle Engkvist Building contractor foundation (210/56).

10. “Meat intake” has been added as a key word.

11. Authors´contributions are now more explained in detail.