Reviewer's report

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

Version: 1 Date: 6 June 2014

Reviewer: Laura Deroma

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Major Compulsory Revisions

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.

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)
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.

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

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.

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.

8) BMI is self-reported (was it really a self-reported BMI or did these women just self-report their height and weight?) 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.

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

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).

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).

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?).

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.

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

15) Was a sample size calculation performed? The authors do not declare how the number of participants was decided.
16) Was the subgroup analysis decided before the study was conducted?

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?

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.

19) Counseling: women were advised “to lower sugar intake to <10 E%”; was this the actual advice or were the woman 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?

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?).

21) Statistical analysis: parametric and non parametric 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.

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.

23) TABLE 1 should be rewritten:

a. Report all P-values (“ns” needs to be avoided)
b. Include the number of women described (“n”)
c. Specify whether the BMI was the one calculated by the researchers or self-reported
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.
Could education be better categorized (does “more than 15 years” mean they have a university degree?), also in order to understand the distribution of education? May be this can be specified in the text? Moreover, a p-value for this comparison is not provided.

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. The subgroup should be described separately.

25) A table showing the (non) 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.

26) Comparisons between supplement users and nonusers should be avoided, since numbers are too low.

27) Supplements with fatty acids paragraph: Leave out the description of statistical differences. Comparisons are not appropriate.

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.

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.

30) TABLE 4: Same comments as above.

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

32) TABLE 6: N and p-values.

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.

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.

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.

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.

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.

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.

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.

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 counselling is programmed, specific information should be given to mothers-to-be on the type of fishes they should prefer when increasing their consumption.

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.

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.

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.

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.

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.

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”

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]”

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.

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

5) Results, Early pregnancy paragraph: when describing mean and standard deviation, please declare the one in bracket is SD, otherwise it is not clear.

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.

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

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?)

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests