Author's response to reviews

Title: Intake ratio of n-3/n-6 PUFAs and risk of breast cancer: a meta-analysis of 274135 adult females from 11 independent prospective studies

Authors:

Bo Yang (ybzu@zju.edu.cn)
Xiao L Ren (rxl@wzmc.edu.cn)
Yuan Q Fu (fuyuqing@163.com)
Jin L Gao (gaojinlong2007@163.com)
Duo Li (duoli@zju.edu.cn)

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Author's response to reviews:

Dear editors and reviewers:

On behalf of my co-authors, we would like to thank the editors and reviewers for your constructive comments concerning our manuscript entitled “Dietary and serum ratio of n-3/n-6 PUFAs and risk of breast cancer: a meta-analysis of 274135 adult females from 11 independent prospective studies” (ID: 1888363717107339). These comments are all valuable and very helpful for revising and improving our paper. We have studied the comments carefully and have made corrections accordingly. Revised parts are marked in red in the paper. The main corrections in the paper and the response to the reviewer’s comments are summarized below:

Editors:

Question: Reviewer 2 has highlighted that your manuscript would benefit from being edited by a native English speaker. Please could you ensure that this is done prior to submitting your revised manuscript?

Response: we will ensure the paper will be edited by a native English speaker before submitting the revised manuscript.

Reviewer #1:

Question 1: did the authors make some efforts to contact investigators of previous studies which were not included in the present meta-analysis? I think it is an important issue and, for each excluded study, the authors should better justify why they did or did not contact the investigators.

Response: Due to Singapore Chinese Health Study (Gago-Dominguez, et al.) and the Netherlands Cohort Study on Diet and Cancer (Laura E Voorrips, et al) reporting association of breast cancer (BC) risk with n-3 and n-6 PUFAs, we had contacted the two authors for data of n-3/n-6 ratio by email. Regarding other references excluded from the present meta-analysis, we did not contact authors, because the original reference only reported relative risk (RR) for association
between n-3 or n-6 and BC risk. Detailed descriptions were shown in line 7#10 of page 5 and Table S1 in revised Additional file 3.

Question 2: it is not clear (despite careful review of supplemental files) what is the exact content of the n-6/n-3 ratio in each study; and apparently no adjustment has been done for some major dietary factors. It would have been, maybe, more astute to simply use the ratio 18:2n-6/18:3n-3. Do you have such data? Regarding the blood ratio, it is important to indicate whether they are phospholipids or total plasma (or serum) as the concentrations of each fatty acid are very different.

Response: As the Reviewer suggested we have made detailed description on n-6/n-3 ratio in line 24-25 of page 5, and performed subgroup analysis by confounding factors adjusted in the revised manuscript (see Table 2). We have also attached importance to ratio of 18:2n-6/18:3n-3, but we have no data provided by prospective studies. Regarding the blood ratio (total serum/plasma or serum phospholipids), we included studies on serum phospholipids (PL) ratio of n-3/n-6 PUFAs, and we will correct the inexact description through the revised text.

Reviewer #2:

Question 1: I would therefore advise against the use of this Newcastle-Ottowa scale to categorize studies, at least for analytical purposes, and be extremely cautious of any subgroup results.

Response: we have only assessed study quality using NOS in the revised text according reviewers’ advice (see line 1#2 of page 8), due to the limitations inherent to such an approach. However, Newcastle-Ottowa scale (NOS) was used for study quality assessment in many published meta-analysis of observational studies. Below are references for NOS used in published meta-analysis:


Question 2: please state the number of studies for each pooled result in the text. The limitation of low numbers should also be prominently described in the discussion section. The substantial overlap of confidence intervals between the subgroup estimates indicates that the differences between countries for instance is not beyond the play of chance, and therefore the conclusions would be tenuous at best. These methods are all implemented in the most recent version of metareg in Stata and could be reported as a sensitivity analysis for metaregression results.
Response: As suggested by the review, we have described the number of studies in subgroup analysis (see subgroup analysis in page 9), and explained the limitation of low numbers of studies in the discussion section (line 10#14 in page 13). Regarding the tenuous and false-positive conclusions from subgroup analysis, we suggested to rephrase the results of subgroup analysis for intake ratio of n-3/n-6, metaregression and permutation test were implemented to test the difference between subgroup and describe corresponding P and adjusted P value in Table 2.

Question 3: Being insensitive to omitting any single study doesn’t really show anything meaningful, in particular it does not strengthen the evidence. Further, this certainly does not have anything to do with showing there is no selection bias (discussion section) – what if all studies have similar selection bias?

Response: Although we included prospective studies concerning serum PL biomarker, these studies were nested case-control studies, in which selection bias form study populations might be unavoidable. After we completed the quality assessment of each original study, we found that occupational exposure populations (e.g., teacher) in study by Chajes (2008) is unlikely to be a perfectly unbiased representative of study population, whereas there was no similar selection bias in other studies concerning serum biomarker. Therefore, considering the reviewer’s suggestion, we rewrited these descriptions in the discussion section (line 15-18 in page 12).

Question 4: The trim and fill method and any test of publication bias will have extremely low power with so few studies, so again the results do not really strengthen the evidence (although again, the converse holds). The method also has some limitations. Just worth being a bit more cautious about all this, as it gives the impression that the authors believe they have demonstrated that there are no possible problems with the analysis.

Response: Regarding the low power from the limited numbers of studies, we suggested that studies on dietary or serum were together analyzed to test publication bias. In addition, we agree with reviewers’ views on some limitations of the trim and fill method, and we removed the trim and fill from the contour-enhanced funnel plot (line 9#12 in page 10; revised Additional file 3). However, contour-enhanced funnel plot in our study was conducted to examine the visual asymmetry of funnel plot, differentiate asymmetry of funnel plot due to publication bias from other factors, and provide a summary effect estimate before and after trim-fill algorithm based on all studies including the estimated missing studies. This statistical method was an updated method of traditional funnel plots, which has been routinely used for meta-analyses. Some evidence for this method is provided below:


Minor Essential Revisions:

1) “RR” appears on page 6 and is not spelled out.
Response: We have made correction in line 25-26 of page 6.

2) What is the “per 1/10 increment” for trends?
Response: per 1/10 increment for trends means per 0.1 increment of n-3/n-6 ratio in diet or serum PL was associated with BC risk.

3) The authors state that a “2-tailed” test was used for heterogeneity; this is either an erroneous detail or something incorrect has been done: chi-squared tests are always one-sided.
Response: We have corrected this error in the statistical section of revised manuscript.

4) “If possible publication bias was found in the meta-analyses, contour-enhanced meta-analysis funnel plot was performed” – why only then? Why not just look at this anyway?
Response: Contour-enhanced meta-analysis funnel plot was performed to explore the source of publication bias if the asymmetry was displayed in traditional funnel plot. If studies appear to be missing in areas of low statistical significance, then it is possible that the asymmetry is due to publication bias. If studies appear to be missing in areas of high statistical significance, then publication bias is a less likely cause of the funnel asymmetry (Figure S2 in revised Additional file 3).

5) What is “significantly inverse evidence”?
Response: It means significantly inverse relationship between dietary ratio and BC risk. We have corrected the similar errors in the revised text.

6) Table 2 and subgroup analysis section: make clear whether these are the highest vs. lowest results or dose-response estimates derived from the Greenland method.
Response: We have made clearer descriptions. (line 4 in page 9 and the title of Table 2).

7) Table 3: Please state the number of studies when some have been excluded in sensitivity analysis.
Response: We have made corrections in the Table 3 of the sensitivity analysis.

We have improved the manuscript based on reviewer’s comments and made some changes in the manuscript. These changes will not alter the framework of the paper. We did not list the detailed changes but marked in red in the revised paper.

We would like to thank the editors and reviewers for their advice and feedback, we hope that the reviewed version will meet the requirements for approval. We look forward to hearing from you.
Yours sincerely,
Duo Li