Reviewer's report

Title: 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

Version: 1 Date: 25 September 2013

Reviewer: Ross Harris

Reviewer's report:

SUMMARY:
This paper describes a meta-analysis of dietary and serum ratio of n-3/n-6 PUFAs and risk of breast cancer. The authors have conducted their study carefully and used advanced meta-analytic techniques to summarise dose-response relationships. The study is generally good, but suffers a little from a low number of studies, and the authors have then perhaps tried to do too much with their data – there are not really enough studies to draw any firm conclusions from subgroup analyses, and they should be more cautious in their interpretation. In particular, I am not sure that the risk differences they describe in USA females for instance is at all conclusive: the authors describe a potential mechanism to explain this difference, but it feels somewhat tenuous. There are also some assertions made about the analysis which do not hold.

Major Compulsory Revisions:
1) The authors have used the Newcastle-Ottowa scale to assess the quality of the included studies, which I have some reservations about. Quality scores have been much criticised in the past, and it is a mystery to me why the Newcastle-Ottowa scale, which has not been peer-reviewed (with somewhat dubious claims of being “validated”) has seen such widespread popularity. I would therefore advise against the use of this scale to categorise studies, at least for analytical purposes, and be extremely cautious of any subgroup results. It is worth reading up on the limitations of this approach and mentioning them in the text – see, e.g.,
   - Cochrane’s documentation on risk of bias
   - Greenland & Rourke. On the bias produced by quality scores in meta-analysis, and a hierarchical view of proposed solutions. Biostatistics 2001

2) Subgroup analyses: please state the number of studies for each pooled result in the text – for most, this is quite low. The limitation of low numbers should also be prominently described in the discussion section – the authors focus a lot on the strengths of their study, and describe a few weaknesses of observational studies in general, but miss out on the key fact of this being a fairly limited pool of
evidence. In particular, the authors may wish to formally test for differences between subgroups using meta-regression. I cannot be sure without checking the data, but 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. Additionally, there are methods that adjust p-values to take into account that significant differences may be obtained from random permutations of the included studies – as a silly example, with 4 studies it might be possible to find a significant difference between studies that were published in odd years vs. even years. See Higgins & Thompson. Controlling the risk of spurious findings from meta-regression. Statist. Med. 2004. 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.

3) There is too much emphasis on the sensitivity analysis excluding one result at a time. Being insensitive to omitting any single study doesn’t really show anything meaningful, in particular it does not strengthen the evidence, only the converse would be true: an observed difference *would* be bad, but no observed difference does not equal good (there is still heterogeneity). 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? Excluding any particular study would of course not yield a better answer.

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 (see http://handbook.cochrane.org/chapter_10/10_4_4_2_trim_and_fill.htm). 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.

Minor Essential Revisions:
1) “RR” appears on page 6 and is not spelled out.

2) What is the “per 1/10 increment” for trends? Please state whether this is a 0.1 increase on the ratio scale or otherwise.

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.

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?

5) What is “significantly inverse evidence”?

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

Discretionary Revisions:
1) The outcome is breast cancer so no need to keep saying that results are for females (e.g., “among USA females” – the reader might almost think there are then some results for males somewhere!)

**Quality of written English:** Not suitable for publication unless extensively edited

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

**Declaration of competing interests:**

I declare that I have no competing interests.