Author’s response to reviews

Title: Childhood fish oil supplementation modifies associations between road density and allergic sensitisation

Authors:
Anna Hansell (a.hansell@imperial.ac.uk)
Ioannis Bakolis (ioannis.bakolis@kcl.ac.uk)
Christine Cowie (christine.cowie@sydney.edu.au)
Elena Belousova (elena.belousova@saxinstitute.org.au)
Kitty Ng (kittykit.ng@hotmail.com)
Christina Weber-Chrysochoou (christinawebber@med.usyd.edu.au)
Warwick Britton (w.britton@centenary.org.au)
Stephen Leeder (stephen.leeder@sydney.edu.au)
Euan Tovey (euan.tovey@sydney.edu.au)
Karen Webb (lkwebb@ucanr.edu)
Brett Toelle (brett.toelle@sydney.edu.au)
Guy Marks (guy.marks@sydney.edu.au)

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COMMENTS FROM Reviewer #1:

In this manuscript Hansell and colleagues examine interactions between fish oil supplementation the first five years of life and traffic related air pollution (TRAP) on allergic disease and lung function outcomes at ages 5 and 8 years.

In the previously published main analyses of the association between TRAP and allergic disease outcomes, the authors showed that TRAP was associated with allergic sensitization to house dust mite and allergic rhinitis (Hansell et al 2014). The present manuscript is a secondary analysis of these data, which is clearly stated in the manuscript.
There are few published studies on the potential interaction between dietary factors and TRAP. Thus, the present study is an interesting addition to the existing literature.

The manuscript is clearly written and easy to follow. The main findings are statistically significant interactions between fish oil supplementation and TRAP for house dust mite, inhalant and all-allergen SPTs and for HDM-specific interleukin-5 response at age 5 years. In contrast, there were no interaction for symptoms of allergic disease.

MAJOR COMMENT

The fact that there seems to be interactions between fish oil supplementation and HDM sensitization but not to symptoms of allergic disease is interesting. Do you think that there is a biological explanation for this difference?

RESPONSE

One of the problems with studying asthma symptoms (breathless, cough and wheeze) is that they may be caused by a heterogeneous range of conditions with different causes. For example, up to six distinct wheezing phenotypes have been suggested from the Tucson study, ALSPAC and other cohorts. Furthermore, symptoms are substantially affected by the use of medications. Hence, it is not particularly surprising the effect of one specific cause for symptoms, tested here, might be difficult to detect. In contrast to symptoms, allergic sensitisation is a less heterogeneous outcome and is not influenced by treatment.

We have added a paragraph on this to the discussion as follows.

"We found interactions between fish oil supplementation and HDM sensitization, and for pre-but not post-bronchodilator FEV1/FVC ratio (in non-movers, i.e. reducing exposure misclassification bias), but not for symptoms. One of the issues with studying asthma symptoms (breathless, cough and wheeze) is that they may be caused by a heterogeneous range of
conditions with different causes. For example, up to six distinct wheezing phenotypes have been suggested (34, 35). Furthermore, symptoms are substantially affected by the use of medications. Hence, it is not particularly surprising the effect of one specific cause for symptoms, as tested here, might be difficult to detect. In contrast to symptoms, allergic sensitisation is a less heterogeneous outcome and is not influenced by treatment."

**COMMENT**

I miss a discussion on the generalizability of the results. It is briefly mentioned in the background section (page 5) that the RCT is conducted in a group of children with a family history of asthma. I think that it may be good to add the description of these inclusion criteria to the description of the study population as well. Could the choice of study population have influenced the observed results, and the generalizability of the results?

**RESPONSE**

Information on recruitment has been added to the methods as follows:

"Recruitment took place before birth – pregnant women whose child would be at high risk of developing asthma, because of a parent or a sibling with a current diagnosis of asthma or with frequent wheeze, were recruited from antenatal clinics of six hospitals in Sydney, Australia (see supplementary methods for more information)."

Further information including the inclusion and exclusion criteria have been added to the supplementary methods.

We agree that the choice of study population may have resulted in selection of a sensitive group most likely to benefit from the intervention and this may not apply to a general population group. The issue of generalizability has been raised in the limitations section:
Further, the RCT was intentionally conducted in children expected to be at higher genetic likelihood of allergic sensitisation because of a family history of asthma, representing a group most likely to benefit from the intervention, but this may limit generalisability.

MINOR COMMENTS

Page 7: It is not entirely clear to me what "nurse-administered questionnaires" means in practice. Where this questionnaires distributed by nurses but filled out by parents (in the clinic or at home), or did the nurse fill out the questionnaire during an interview with the parents?

RESPONSE

The latter – we have changed ‘nurse-administered questionnaires’ in the methods and supplementary methods to:

"Questionnaires were administered by nurses and obtained information on…"

COMMENTS Reviewer #2: This study presents a challenging issue on the effect of intervention of fish oil supplementation during childhood on the risk of asthma development from traffic-related air pollution. The manuscript is well written and in spite of strong limitation of small sample size and potential misclassification of the TRAP, effect of intervention is clear. I would agree on the publication of this paper in the journal. I would like to recommend the following issues need to be described and in the main text and discussion.

1. The 616 final subjects were recruited out of 2095 eligible participants and at the end of follow up, only 399 participants were left. Please discuss the potential selection bias issue in the recruitment and loss of follow up of the initial RCT participants.
RESPONSE

We consider that the recruitment issue is unlikely to introduce directional bias – the child’s allergic status was unknown at recruitment and those invited to participate were selected to have children at higher risk of allergic disease. We have shown that loss to follow-up at ages 5 and age 8 years was not related to traffic exposure (weighted road density), but it was related to socio-economic status (those with lower SES were more likely to drop out). If lower SES was associated with worse diet, these participants might be expected to have benefitted more from fish oil supplementation, and this would have led to bias towards the null.

We have added the following to the discussion limitations section.

"Just under a third of eligible pregnant mothers agreed to participate in the original RCT, however, this decision could not have been influenced by the child’s as yet unknown asthma or allergic status. A further quarter of children had dropped out of the cohort by age 8 years; this was not related to exposure (WRD), but children who dropped out were more likely to have parents without a university education (16). The latter might introduce bias that would reduce the size of an observed effect, as children of lower socio-economic status may be more likely to have a diet with a lower omega-3/omega-6 ratio, therefore benefit more from fish oil supplementation."

We acknowledge that we do not have data from those who refused to take part in the RCT. However, as we already state as the last line of our conclusions “This important interaction needs confirmation in independent cohorts and randomized trials, but the findings highlight the importance of studying environment-environment interactions.”
COMMENT

2. It is suggested to include a discussion on the role of epigenetics, i.e., potential effect of fish oil supplementation on the modulation of DNA methylation with the TRAP-related asthma development in the discussion section.

RESPONSE

We thank the reviewer for this suggestion. We have added the following new paragraph to the discussion:

"Epigenetics may help provide a mechanism explaining the findings from this study. Traffic related air pollution has been shown to induce changes in methylation levels in genes relevant to asthma and allergic sensitisation (26, 27) and histone H3 modification (28), probably by increasing oxidative stress and pro-inflammatory responses. Epigenetic variations near and through the fatty acid desaturase (FADS) gene cluster account for variations in circulating and cellular long-chain PUFAs, the bioactive metabolites synthesised from dietary PUFAs (29). Supplementation with dietary n-3 PUFA has been shown to be associated with DNA methylation of PUFA biosynthesis genes (30), leading to gene silencing of inflammatory pathways (31). In this study, the fatty acid dietary supplementation occurred during the first five years of life which coincides with a critical time in development of the immune system, therefore a hypothesis from our findings is that epigenetic modification induced by exposure to traffic related air pollution was protected against by epigenetic changes in children who received the dietary intervention. "