Author's response to reviews

Title: Antioxidant-rich dietary intervention for improving asthma control in pregnancies complicated by asthma: study protocol for a randomized controlled trial.

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Author's response to reviews: see over
To the editor:
Please find below our amendments to the manuscript.

**Reviewer's report:**
Major Compulsory Revisions/Responses

1) There is a discrepancy between the Abstract (mild, moderate or severe asthma) and p7 (mild or moderate/severe), so clarification is required as to whether three categories of asthma severity or two will be used.
We have corrected this to: mild, moderate or severe asthma.

2) I would suggest that there are too many references, some being unnecessary, such as a textbook reference (#2) to a definition of asthma. Is #5 an appropriate citation -is this not a secondhand opinion rather than reference to first hand experimental evidence (of 'a high level of oxidative stress in the lung')? There might be some attention to the quality of some of the literature cited, and, use of 'e.g.' to cite one, most appropriate reference, rather than several. One might also question why, for example, #62-66 are cited for the first time in the Discussion, and have not been previously introduced (i.e. in the Introduction/Background).
Thankyou for this suggestion. We have taken out the textbook reference (#2), as well as reference #5 by Killeen et al and we have gone through the manuscript and taken out a further 14 references.

We have moved the sentence in the discussion: *The lungs have endogenous antioxidant mechanisms to combat the damaging effects of reactive oxygen species; however levels of antioxidants in the lungs as well as in circulation are reduced in asthmatic patients [5-7] into the introduction.*

3) Methods (p8) -clarity required on whether the booklet 'healthy eating during pregnancy and lactation' intended for use in the control arm is the same as the 'Australian Guide to Healthy Eating for pregnancy' intended for the Intervention arm, and if not the same booklet, how can a Control arm participant not receive the current national Guide?
In the protocol section, we have further explained the information contained in the standard dietary care booklet for pregnant women at Lyell McEwin Hospital and have separated out what happens in the control vs. intervention group. All women will receive the standard care pregnancy booklet.

**Protocol**

At ~12 weeks gestation, asthmatic women will be recruited to participate (*Figure 1*) in the 12 week intervention study, with an initial 4 week run-in period (*Table 1*). At this initial visit, women will have the study procedures explained and have their asthma assessed (described below). A telephone call to determine consent will be made at ~13 weeks gestation in which a date to start the subsequent run-in period will be made. Signed consent forms will be obtained at the 14 weeks gestation visit. At this visit, the run-in period will commence where the women will be provided individual asthma management and education, complete a questionnaire on how/if their food intake has changed since learning they were pregnant, and will complete a food frequency questionnaire (FFQ). At 18 weeks gestation, women will be randomized into a control or intervention group (i.e. baseline visit) (*Table 1*). At this baseline visit, weight and blood pressure will be measured, a fetal scan performed, questionnaires on asthma control, physical activity, and smoking will be completed, a blood sample taken, and dietary information collected (i.e. 24-hour recall, food group count). These same clinic visit
measurements will occur at 24 and 30 weeks gestation for all women. Phone calls will be made at 20, 22, 26 and 28 weeks gestation for information on asthma control and dietary intakes, and women in the intervention group will receive additional dietary counselling and support regarding the consumption of antioxidant-rich foods. At 30 weeks gestation, both groups will come back to the clinic for their final visit (Table 1). At delivery, birth outcomes will be assessed.

**The control group** will receive only the standard care Lyell McEwin Hospital pregnancy booklet on “Healthy eating during pregnancy”. The booklet contains the recommended number of serves of each food group to be consumed in pregnancy, according to the 2013 Australian Dietary Guidelines (http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/n55a-australian_dietary_guidelines_summary_131014.pdf), as well as further information on key nutrients during pregnancy, supplements, weight gain during pregnancy, and guidance on for example, alcohol, caffeine and water intake, and listeria, as referenced from SA Health; RANZCOG 2009; Noarlunga Health Services; and Food Standards, Australia and New Zealand. No additional dietary education will be provided to the control group.

**The intervention group** will receive the same booklet however will be further counselled on types of antioxidant-rich foods to purchase and consume such as fruits (for increasing intake of carotenoids, vitamins A and C), vegetables (carotenoids, vitamins A and C), wholegrains (vitamin E, selenium) and lean meat (zinc, iron, long chain omega 3 fatty acids) over the 12 week study. Examples of types of foods to consume will also be provided: e.g., choosing spinach leaves instead of lettuce, sweet potato instead of white potato. Women will be provided with a list of foods that are high in antioxidants and will be asked to identify which types of these foods they are most likely to purchase and consume over the 12 weeks. Women in this group will also be given meal/snack suggestions to assist compliance. Previous pilot work from the authors has identified, in a dietary pattern analysis, that a high fat/sugar/take-away pattern was evident in this population of pregnant women with high consumption of take-away foods, crisps, refined grains and cakes and limited consumption of fruits and vegetables. Therefore, a shopping voucher of $30 per week per participant will be provided which will contribute to the cost spent on fruits, vegetables, as well as wholegrains and lean meats.

4) It would seem that Consent is being obtained at the same time/occasion as the participant being invited to take part in the study (p9). This contravenes at least good practice, if not ethical protocol, and participants would normally be given some time to consider their involvement after being informed, normally at least a day. In practice, one can see that participation in the trial only begins at a later date, by which time, the participant can withdraw, but nevertheless, the principle might be addressed or the timing of consent being obtained made more clear.

Thankyou for you this. We have clarified in the protocol section when signed consent will be obtained: A telephone call to determine consent will be made at ~13 weeks gestation in which a date to start the subsequent run-in period will be made. Signed consent forms will be obtained at 14 weeks gestation visit.

5) The randomisation is complex, and it is not clear how stratification can take place based on so many outcome measures (parity, asthma severity, and age, body mass index and vegetable intake) -greater detail should be given as to how this will be done, whether by an independent statistician and/or whether this procedure is computer driven, and what service/software will be doing this.
We have amended the randomisation to: *The randomisation schedule will be created by an independent statistician using ralloc.ado version 3.6.1 in Stata version 11.1. Randomisation will be stratified by pre-pregnancy asthma severity (based on GINA guidelines) and body mass index.*

6) There needs to be comparability between a) the strategies that achieved 22% dropout (wouldn't this be better reported as 78% retention?) and 80% compliance (p12) and b) the sample size discussion (p13) which talks about 'a 10% loss during the run-in period' - why wouldn't a 22% loss be estimated?, and 'a further 25% loss during the intervention period' - why wouldn't a 20% loss be estimated?

Thankyou for this comment. The previous trial had an overall 22% loss across the duration of the trial and 20% of completers were excluded from the analysis due to non-compliance. We have removed the sentence in the compliance section stating we achieved a 22% drop out and 80% compliance rates.

We have revised the paragraph on sample size to state: *The previous trial achieved 78% retention; however 20% were removed from the analysis due to non-compliance. Therefore, we will recruit 168 women so that 104 women (52 per group) will complete the study and be included in the analysis.*

7) In the Discussion, health expenditure on asthma is raised, however, there do not appear to be any health economic outcome measures. There will be economic consequences for the participants (and despite the authors mentioning that fast food is expensive, one barrier to compliance is potentially the high cost of fresh fruit and vegetables, and there is no financial recompense for participants in the trial for this). Furthermore, there does not appear to be any attempt to assess the cost of the intervention/any cost-benefit analysis.

We have removed the sentence on economic consequences; however we appreciate that we could do this in future studies when we demonstrate the intervention is successful.

8) Perhaps little can be done about the design, but it seems that if the trial is to address asthma control in pregnancy, virtually half of the pregnancy is missed before the Randomisation/Intervention begins to be delivered at 18 weeks.

We appreciate this comment. We have decided on this time frame as most women attending Lyell McEwin Hospital attend their first antenatal visit at around 13 weeks gestation. Therefore, capturing pregnant women prior to this is difficult. The senior author on this paper has also reported on correlations between various antioxidants and birth weight centile in women with mild asthma at 30 weeks of gestation. This suggests mid gestation may be an appropriate time to intervene with antioxidant rich foods to support fetal growth and asthma outcomes.

Editorial requests:
1) If applicable, please include an acknowledgement section at the end of the manuscript before the reference list. Please acknowledge anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship.

Please also include the source(s) of funding for all authors. Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements. Please state clearly whether or not you have funding in the acknowledgement section. If there is no funding,
We have no current financial support for this study.

2) Please include a figure title and legend section after the reference list. The figures should not be included in the main body of the manuscript.

We have included the figure title and legend after the references. We have deleted the figure title from the actual figure.

PLEASE SEE OUR OWN FURTHER CHANGES TO THE MANUSCRIPT BELOW:

**We have modified the “background” section in the abstract to include:
Allowing for a 78% retention including a 20% removal of women from the analysis due to non-compliance, we will recruit a total of 168 women.

**We have included a few sentences on bioavailable zinc in the introduction:
Other human mechanistic studies have shown that the airway epithelium in asthmatic adults is more susceptible to oxidants than in non-asthmatic adults [8], in which work by Zalewski et al identified lower labile sputum zinc levels was associated with increased frequency of wheeze, asthma severity and reduced lung function [9].

**We have also amended the section on “dietary measurements”. We are now including a questionnaire that picks up any dietary changes that may have occurred since identifying the women became pregnant:

**Dietary measurements**
At 14 weeks gestation (run-in phase) all women will firstly complete a questionnaire on pregnancy food intake. This questionnaire will capture information on how/if they have changed their diet since identifying they are pregnant. Weeks gestation will be recorded for both the time they found out they were pregnant and current gestation. Questions on how they have changed their diet will be asked including reference to each of the food groups in the Australian Dietary Guidelines, plus alcohol consumption, soft cheese and deli meat consumption (listeria), fish consumption, and intake of non-core foods such as take-away foods. Next, all women will complete the standard Cancer Council of Victoria’s Dietary Questionnaire for Epidemiological Studies FFQ to assess food intake 1 year prior to pregnancy. A 24-hr recall will be administered at the face-to-face visits and over the phone. This questionnaire asks for detailed information on meals and snacks consumed 24hr prior to the clinic visit. Data will be entered into the Foodworks (Xyris, Brisbane) database. Throughout the 12 week study, all women will be required to complete a weekly count of the average number of serves of each food group consumed according to the Australian Dietary Guidelines. Eating habits will be measured with using the Eating Habits subscale from the Project EAT Survey [47]. All items are scored on a scale of 1 to 5 and summed; a higher score indicating less desirable eating habits.