Author’s response to reviews

Title: Effect of continued folic acid supplementation beyond the first trimester of pregnancy on cognitive performance in the child: a follow-up study from a randomized controlled trial (FASSTT Offspring trial)

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Author’s response to reviews:

Dear Dr Lee,

Thank you for your communication of August 28th 2019 regarding our manuscript “Effect of continued folic acid supplementation beyond the first trimester of pregnancy on cognitive performance in the child: a follow-up study from a randomized controlled trial (FASSTT Offspring trial)”. We appreciate your time and effort in reviewing our manuscript and acknowledge your valuable feedback.

[Rest of the response continues with the author's response to the reviewer's comments, which are not visible in the provided text.]
We very much appreciate the opportunity to address the comments from the editors and reviewers. We have now revised the manuscript in line with the comments received. We are submitting the revised manuscript with corrected text highlighted.

Please find in the table below a point-by-point response to each of the comments received, which are included verbatim.

We confirm that all author details on the revised manuscript are correct, that all authors have agreed to authorship and order of authorship, and that all authors have the appropriate permissions and rights to the reported data.

We are very grateful for your valuable comments and the time and effort involved in reviewing our work. We hope that the revised manuscript will meet with the approval of the editors and reviewers.

Thank you very much for your input so far and the opportunity to have our manuscript reconsidered.

Yours sincerely,

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Requests from Reviewer #1 - Regan Bailey

This manuscript presents RCT data on the cognitive testing of the children of mothers who were randomized to receive folic acid dietary supplements after the first trimester or not. 70 children are reported on at year 7 and 39 children at age 3. Multiple cognitive measures are used. Folic acid DS are recommended during the first trimester to prevent NTDs, but it is
largely unknown whether continued supplementation is warranted, and observational studies have either demonstrated a positive or null association with continued supplementation and cognitive outcomes. Limited RCT data exist.

The study is well designed. Compliance and follow-up were addressed to the extent possible. Power calculations were employed and appropriate. The paper is quite well written and easy to follow. The authors have provided a comprehensive review of the existing literature in the introduction and discussion. Very minor edits or clarifications outlined below. Response: We thank Dr Bailey for these positive comments.

Line 119 - specify when consent was obtained, listed over the phone and assuming at the visit, but clarity would be helpful here.

Response: In compliance with ethical requirements, initial verbal consent was received over the phone at the time of re-contacting the mother for the follow-up investigation of her child at 3 and 7 years. After the mother had provisionally agreed (over the phone), signed written consent from the mother and assent from the child were obtained at the time of the appointment. We have revised the text in the Methods section to clarify these details: Methods Page 5, lines 112-117

Genetic data were obtained but not presented here, why?

Response: Apart from reporting MTHFR genotype for both mother and child (Table 2), the genetic data are not presented as they are not relevant to the current paper. Epigenetic data on this cohort were however the subject of two previous publications - Caffrey et al 2018 (ref 43) and Irwin et al 2019 (Clinical Epigenetics 2019; 31). In response to this comment, the latter paper is now added as an additional reference (ref 44) in the current manuscript. We thank the reviewer for drawing our attention to this matter and giving us the opportunity to add an important relevant reference dealing with genetic data on this cohort. Revisions: Discussion, Page 16 line 346. New ref 44

Under the limitations, please add that some of the same children were measured at both 3 and 7 years

Response: We provide a ‘matching sample’ for 50% of children who were sampled at both time points - 34 of 70 children sampled at 7 years were also sampled at 3 years. From the
other perspective, of the 39 children sampled at 3 years, 34 also contributed to the sample at 7 years (i.e. only 5 were lost to a second follow-up); these, along with 36 other children who had not been sampled at 3 years, provided a total of 70 children sampled at 7 years.

We have clarified the text as succinctly as possible in the relevant place in the Discussion.

Revisions: Discussion, Page 17, lines 377-379

Were any adverse events reported in study?

Response: We can confirm that no adverse events were reported at any time during the original FASSTT trial or at either of the follow-up phases the study. We have now added a sentence to this effect in the opening paragraph of the Results section.

Revisions: Results Page 8-9, lines 194-196

Requests from Reviewer #2 - Nancy Potischman

T2 and T3 Folic acid - cognition This is a well-conceived and executed study of folic acid exposure in the second and third trimesters on offspring cognition compared with exposure only in the first trimester. It will make a large impact in the field due to the clear findings and strong study design. It has many positive attributes and could not be done in many locations where folic acid fortification would influence status to a considerable degree. Despite the small sample size, and marginal response rate, the comparisons with other data outside of their study adds confidence to the findings on cognition. The impact of maternal folic acid status during the pregnancy predicting the outcomes, after adjustment for important early confounding factors, adds to the confidence in the findings. Response: We very much appreciate these positive comments from Dr Potischman.

It was interesting to see an effect of breastfeeding and more detail about the length of breastfeeding and perhaps other factors related to this variable would be of interest.

Response: We agree that these details would be of interest. Unfortunately, however, we did not record the duration and other details related to breastfeeding. At the time of follow-up of the children at age 3 and 7 years, the question was simply asked as to whether the mother had breastfed her baby or not.
As an outsider to this field on cognition, it would have been helpful to have all reviews of the literature in Background and Discussion be pertinent to the timing in the pregnancy. For example, Page 4 line 72, could the null finding be due to the short duration of folic acid exposure (8 weeks)? Page 14, references 33, 34 are first trimester only - should more be said about reference 35 relevant to timing? A more critical review of this literature would be helpful to the reader.

Response: We very much agree that the timing of exposure to folic acid within the pregnancy is an important consideration as regards cognitive outcomes in the child, and that we need to be clearer on this when reviewing the literature. In the Background and Discussion, we have revised the text in the relevant places to be clearer on the period of pregnancy that we are referring to.

In amending the Discussion to address the reviewer’s important point about timing, however, we made the decision to remove the original reference 35 and the related sentence. This paper provided a systematic review of 13 observational studies, but on reflection, as a review article it is not as helpful to the reader as the original articles in terms of summarising the current literature linking maternal folate at particular time points of pregnancy with child cognition. This is primarily because of major differences between individual studies included in the review in relation to the timing of maternal folate exposure, in that mothers were sampled across all three trimesters of pregnancy.

The removal of this reference and related text, together with the small but important revisions to the remaining text, have improved the clarity of our review of the evidence with respect to the timing of folic acid in the pregnancy. We thank the reviewer for raising this issue and hope that the revised text clarifies the matter.

Revisions: Background Page 3-4: Lines 65-74; Lines 75-81; Lines 86-87. Discussion Page 14, lines 302-310

Page 3, line 62 - It would be of interest to know what form of folate was found in the brain. Is it folic acid or a metabolite?

Response: We can confirm that in this context we are referring to total folate rather than a particular folate derivative. This is now clarified in the revised text and we have also supported this statement by the inclusion of an additional reference (new reference 13), along with the existing one (reference 12). Revisions: Background Page 3, Line 60-62. New Reference 13
is there an impact of having non-fasting samples? If so, this could be mentioned in the discussion.

Response: Fasting blood samples would be unusual in pregnancy studies, and may in fact cause problems for ethics committees at the time of seeking ethical approval. However, we would not expect any impact of using non-fasting samples in the current study because our main conclusions in relation to blood folate responses to FA intervention (maternal and in newborns) are based on red blood cell (RBC) folate (albeit we also present serum folate values). The advantage of RBC folate, in contrast to serum or plasma folate, is that it provides a long term measure of folate biomarker status and is unaffected by recent intake. We have added a sentence to this effect and incorporated a supporting reference in the revised Discussion.

Revisions: Discussion Page 16, lines 363-365. New ref 48

Table 1 - granted there were fewer people in the Placebo group at the age 3 testing, but this group did seem slightly different than the folic acid group. Is it worth mentioning even if not significant?

Response: Our title, aim and conclusions relate to offspring cognition at 7 years. The assessment of the child at 3 years was performed only in a sub-sample and its primary purpose in the current paper was to provide pilot data for the main child assessment at age 7 years. For this reason, we do not feel that it is appropriate to mention these non-significant differences between the treatment and placebo group at age 3 years.

Figure 2 - does not add much more than what has been already presented. Is this a common way to evaluate such data so it adds more than what is in the table?

Response: We feel that this figure does provide important data which adds to what is already presented in tables, in that it shows in a visual way that the cognitive outcomes in children sampled at 7 years are generally supported by the pilot data from the same children sampled at 3 years, and the broad agreement in results at both time points contributes some degree of internal validation to our findings. Nowhere else in the paper is there an opportunity to visualize the consistency of cognitive results according to treatment allocation of the mother as the child develops. Also this is the only place in the paper where cognitive data are split by sex; this is important as sex differences in cognitive child development are well described in the literature. This general approach has been used in other papers examining child cognition.
over follow-up periods, including one paper in this journal (Conroy et al BMC Medicine (2019) 17:98).

For these reasons, we do wish to retain Figure 2 and hope that the reviewer now agrees with this position. We have however redrawn the figure and revised the footnote to improve the clarity of what we are presenting.

Revisions: Figure 2

Page 16 - reference 44 - does this study directly address potential adverse effects of unmetabolized FA? There are studies that discuss potential effects, though the evidence of impact is weak. Unmetabolized FA is found in many individuals, even with marginal folate status, it is only found in large amounts at very high levels of folic acid intake and folate status. The authors should consider the wording on this topic carefully, though their discussion of dose and prudent recommendation are appropriate and appreciated.

Response: We agree that we were unclear in the wording used when commenting on reference 44, and accept that this paper does not provide evidence of adverse effects. We hope that the reviewer will be happy with our revised text.

Revision: Discussion Page 16, lines 348-351