Author’s response to reviews

Title: Prenatal Micronutrient Supplementation and Postpartum Depressive Symptoms in a Pregnancy Cohort

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Author’s response to reviews: see over
Authors’ response to Reviewer #1

ABSTRACT
Discretionary Revisions
1) Methods: Consider including sample size used for current analysis in the abstract.

RESPONSE: As stated in the text: “475 participants…. completed the EPDS at least twice in pregnancy and at 12 weeks postpartum, 416 (88%) scored <10 and 59 (12%) scored ≥10”.

2) Results:
1st sentence: Consider incorporating what being < 10 and ≥ 10 on the EPDS represents into the sentence. Some readers may not be familiar with the EPDS and it’s typical cut-offs (e.g. what might represent “low” or “high” depressive symptoms).

RESPONSE: Thank you, this change will enhance clarity. We have revised it as suggested.

3) 2nd sentence: Consider defining “most” (e.g. x/x examined) and adding word “mean” before intakes.

RESPONSE: Revised as suggested.

4) 2nd and 3rd sentence: For clarity, consider stating that these associations are based on bivariate analyses.

RESPONSE: Revised as suggested.

INTRODUCTION
Discretionary Revisions
5) 1st paragraph, line 8-10: Consider replacing “mental (and physical)” with “health and development” and adding reference to at least one health effect.

RESPONSE: Revised as suggested; references were given in the next sentence.

6) 3rd paragraph, line 4: Consider replacing word “pregnancy” with “the offspring”.

RESPONSE: Revised as suggested.

Minor Essential Revisions
7) Consider adding your initial hypothesis (hypotheses) to end of the introduction.

RESPONSE: Although the suggestion is consistent with some practices, in this particular manuscript we feel this is unnecessary as the purpose states clearly the intention of this study.
METHODS AND MATERIALS
• Study design and participants
Discretionary Revision
8) 2nd sentence: Does “drug abuse” included “alcohol abuse” Might clarify.

RESPONSE: Thank you; we have clarified this.

9) Last sentence: If possible, consider referencing a previous APrON publication, as well as their website.

RESPONSE: Revised as suggested; the publication is currently in press, thus the full citation is not yet available. The APrON website was also given in the text.

• Participant background and covariates
Minor Essential Revisions
10) Nice description of demographic and social/lifestyle variables in Table 1. For clarity re: direction of findings, consider showing column rather than row percentages (e.g. % of mother in each EPDS group with 3 chronic diseases, etc).

RESPONSE: Thank you for the positive comment. The data was presented in rows to reflect the longitudinal nature of the study (based on recommendation from the APrON biostatistician).

11) Consider adding footnote re: definition of EPDS and cut-offs.

RESPONSE: Revised as suggested: EPDS≥10 = “at least probable minor depression”.

Major Compulsory Revision
12) Do you have data on the nutritional status of these women during pregnancy (e.g. weight status, weight gain, iron/anemia status, etc)? If so consider exploring as moderator (e.g. did the effect of MN supplementation on PPD vary by prenatal nutritional status). If not, consider addressing in discussion section as limitation.

RESPONSE: We do not have nutritional status data available for analysis at this time. We recognized this limitation and addressed it in the discussion section (please refer to pages 14-15). The reviewer's suggestions pinpoint some important research questions which we will surely analyse in the future when those data are available.

• Prenatal nutrient intake form supplements –SIQ
Major Compulsory Revisions
14) 4th paragraph: Consider adding justification for method used to assess individual supplement intakes. Have others validated method, etc? In discussion, consider addressing its limitations and expanding upon alternative (potentially more valid/reliable) methods.
RESPONSE: The SIQ was based on validated tools used in previous studies (Centers for Disease Control and Prevention, 2006, Csizmadi et al., 2007, Statistics Canada, 2004) and was adapted for use with pregnant women. Supplements were verified (where possible) to corresponding natural product number (NPN) or drug identification number (DIN). Health Canada assigns a NPN or a DIN to all NHPs that have met its standards for quality, safety and efficacy (Health Canada, 2003). NPNs and DINs were used to identify products and their formulations were entered into the APrON database. For products that did not have a NPN or DIN, ingredients were recorded from product labels and where necessary from manufacturer/supplier websites. Due to the detailed recording of the supplement intake, by getting labels/containers, and verifying ingredients, we believed this is the most reliable and valid method in capturing the data.

The text has been revised to read: “Where possible, the products were verified using their NPNs and DINs against those within Health Canada’s Natural Health Products Database, to confirm their formulations; then they were entered into the APrON database. A NPN or a DIN is assigned to all NHPs that have met its standards for quality, safety and efficacy by Health Canada Products that did not have a NPN or DIN were verified by obtaining ingredients from product labels or by finding the information from the manufacturer/supplier websites.”

We also have added to the discussion section (page 15, paragraph 1, last sentence): “we acknowledge a major limitation with the SIQ is self-reporting; thus we did not know whether the women actually ingest their supplements as they said they did.”

15) 4th paragraph: The average intake of each individual nutrient intake across 3 time points was examined in the current analysis. Did you examine timing or dose effects (e.g. taken at all three time points vs. just twice, during 3rd trimester and postpartum vs. 2nd and 3rd trimester). If not, consider exploring/adding to results.

RESPONSE: This analysis was done for another publication by another author (manuscript to be submitted), and thus those findings are not provided in this manuscript. Her findings in the current draft manuscript reported “Small but significant differences were observed in the reported intake of multivitamin/mineral supplements between trimesters, whereby 97% of participants reported their use in the first trimester, and 92% in the third trimester (p=0.01). Many women also reported taking single nutrient supplements, and this varied by trimester with 45% indicating their use during the first trimester, and 55% by the third trimester (p=0.02).”

Minor Essential Revisions
16) Last sentence of 4th paragraph: Consider adding reference for IOM.

RESPONSE: Revised as suggested.

Statistical Analysis
Major Compulsory Revisions
17) Did you examine interactions between nutrients (e.g. iron and vitamin C; iron and zinc, etc)? If not, consider adding to your analysis or mentioning in discussion as limitation/direction for future study.

RESPONSE: Thank you for the suggestion. There is no current literature to support the assessment of interactions as related to PPD; thus further analysis for interaction would be data mining without scientific rationale or a priori hypotheses for specific nutrient interactions. While we thank the reviewer for this suggestion, we believe it is not warranted within the context of this manuscript to examine interactions. However, we acknowledge this limitation and have added it to the discussion section, as follows: “A limitation in the analysis was that we did not assess for interaction among the nutrients due to absence of a priori rationale from the literature. One type of future research to consider would be to assess whether specific nutrients interact to impact postpartum depression.”

RESULTS
• Nutrient data
  Discretionary Revision
  18) Last sentence: Consider clarifying here that overall trend was not statistically significant.

RESPONSE: Revised as suggested.

• Assessing predictors of depressive symptoms on the EPDS
  Discretionary Revisions
  19) 2nd paragraph: Although not significant, consider adding statistic for omega-3. Even though it is not significant, direction of association would be informative.

RESPONSE: Revised as suggested.

DISCUSSION
Minor Essential Revision
  20) 1st paragraph, line 12: Consider adding references after sentence that ends with “associated with depression”

RESPONSE: Revised as suggested.

21) 2nd paragraph: Good description of previous findings. Consider being more specific re: how selenium status was assessed for each study described. For example, in the second to last sentence of the paragraph, it is not clear if selenium status was based on dietary/supplement intakes or serum concentrations.

RESPONSE: Thank you for the comment. We have revised as suggested: the word “supplement” was added to the findings from Rayman.

Discretionary Revision
22) 5th and 6th paragraphs: For increased focus, consider shortening/combining these 2 paragraphs.

RESPONSE: We appreciate the suggestion, but we feel these two paragraphs cover important points, and thus should be presented as is.

Major Compulsory Revisions
23) 7th paragraph: Did you examine serum concentration level for any nutrient. If not, you might consider stating that it was an overall limitation to your nutrient analysis (not just for selenium).

RESPONSE: Nutrient assays were not available at the time of this manuscript; this limitation has been stated in the discussion section: “Another limitation is that the present study did not have access to serum levels of selenium or any of the other nutrients discussed in this paper, thus there is no information as to whether supplementation affected biological levels.” We would like to point out, however, that a very recent case-control study also found a significant association of selenium intake and mood, with no data available from serum levels ---- this information has been added to the Discussion: “A recent nested case-control study by Pasco and colleagues found low intake of selenium (<8.9 μg/MJ/day) was associated with almost a three-fold increase in the likelihood of major depressive disorder (OR 2.95, 95%CI 1.00-8.72) after adjusting for age and socio-economic status [45].”

24) 9th paragraph: Consider adding more re: implications for further research (e.g. how can future research build on your findings (e.g. use of objective measures to assess nutrient status, looking at interactions between nutrient intakes/status, etc.).

RESPONSE: Revised as suggested: “Implications for future research include using nutrient status (when assays from APrON are available) to assess the relationship between blood nutrient levels and mood, as well as investigating possible interactions amongst nutrients and their effect on mood.”

Authors’ response to Reviewer #2
25) The ms describes the relation between symptoms of postpartum depression and prenatal supplement intake. The writing is clear although it is quite broad in scope. The methodology is not clear. For example, it is not clear how the intake of supplements was calculated; it is stated that the authors took the number of times the question was asked and the dose and then divided the number of queries. It is not clear how this relates to supplement use.

RESPONSE: We are puzzled as to how the methodology could be more clearly stated than it currently is on pages 6 – 8, where we presented the development and application of the SIQ, the recording of the supplement intake, as well as the
calculation of the individual nutrients obtained through supplements. As we say on those pages, “The structure of the SIQ was adapted from questionnaires used in other studies of supplement intake, such as the National Cancer Institute and the Canadian Community Health Survey…….. A trained Research Assistant (RA) conducted the interview with each woman to elicit the information. All information provided by the participants was reviewed with them to ensure the items were correctly recorded. Women were also asked to bring in bottles and other containers of the supplements they were taking. Brand names, individual nutrients and their amounts, as well as dosage (i.e. number of pills/capsules/tablets taken per day) were recorded……. To obtain an average intake of each nutrient in the supplements, reported intake was averaged over the number of times data were collected. For example, if information on vitamin D supplement intake was collected at each trimester (i.e. three times), then the total amount consumed was divided by three; if information was collected at only two times, then the total was divided by two.”

26) It is also not clear how the authors could disentangle the intakes of various nutrients in supplements when most women take a prenatal multinutrient supplement.

RESPONSE: Women took multi-nutrients of various combinations. There were over 400 brands of supplements taken by the participants in our sample, thus we recorded individual ingredients in each brand of supplement taken, and verified them using the Health Canada Natural Health Products database or manufacturer’s ingredients listing (refer to text page 7, 1st paragraph). Thus we were able to disentangle the individual nutrients taken by the participants. For example, while some nutrients were highly correlated (e.g. B vitamins), other nutrients such as selenium and omega 3 were not consistently included in the various brands of supplements, and the types of nutrients taken varied by participant.

27) The authors have taken multiple tests and find an association with selenium which may be a chance finding. This is not dealt with.

RESPONSE: The following statement has been added to the Methods section in reference to bivariate analyses: “Bonferroni correction was used for multiple test comparisons.” Furthermore, we did not rely solely on bivariate analyses to assess the predictors against the outcome. Multivariate analyses, which by definition adjust for covariates, were sufficiently robust to justify our conclusion that our findings are not due to chance. In addition, as discussed in the text, our findings on selenium are consistent with the world literature.

28) The writing should focused in on essential information. Temporality is not clear, just because depression was assessed postpartum only.

RESPONSE: We think our writing was focused and presented the essential information related to our topic of prenatal micronutrient supplementation and PPD. We are especially concerned that this reviewer was unclear about temporality, as we think it was specifically detailed in the methods section, whereby supplements were measured during pregnancy, and PPD was measured postpartum. Furthermore, temporality is clearly demonstrated in Table 2, where women below RDA are more
likely to have EPDS ≥ 10 compared to those at or above RDA (see horizontal output in Table 2).

Authors’ response to Editor's comments:
29) "Authors are requested to remove all the unconventional abbreviations used in the manuscript.

RESPONSE: The following abbreviations have been removed: PPD, SIQ, BornC, RAs

30) The results section has numerous sentences describing the methods for the regression analysis and models, which should all be moved to the methods section.

RESPONSE: We often find reviewers disagree on how much of the analysis approach should be in the Methods vs at the beginning of the Results, but we are happy to revise as suggested: the section on assessing participants with missing data was moved to Methods (before Statistical Analysis section); all details about variables included in models one to four have been removed from the Results section and placed in the Methods section.

31) Because measurements of EPDS done in pregnancy are likely to be strongly correlated, the issue of multicollinearity may exist; provide some information regarding the fit of the model.

RESPONSE: We tested the fit of the model with Hosmer and Lemeshow's goodness-of-fit test, where Hosmer-Lemeshow chi2(7) = 8.08, and p = 0.33, indicating that our model fits the data well. For regression diagnostics, we computed the variance inflation factor (VIF) to test for collinearity; the mean VIF = 1.13, with VIF for individual variable ranged from 1.03 to 1.17 (and tolerance from .84 to .97), indicating that multicollinearity is not likely to be a problem in the model. These tests and findings have been added to the text under the Methods and Results sections.

32) The sample size seems a limiting factor; please provide estimates of detectable ORs or do some power calculations for the SS used in the analysis.

RESPONSE: We conducted a power analysis with the following assumptions:
- The prevalence of PPD is 12% in the population
- Our projected relative risk (RR) of 1.5 for depression in those below RDA compared to those at or above RDA. Thus,

<table>
<thead>
<tr>
<th>Nutrient intake</th>
<th>Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below RDA</td>
<td>18%</td>
</tr>
<tr>
<td>At/above RDA</td>
<td>12%</td>
</tr>
</tbody>
</table>
RR = \%\text{depressed (II)} = 0.18 = 1.5
\%\text{depressed (AI)} = 0.12

Using the “powerlog” STATA11 command for logistic regression, we have the following output:

<table>
<thead>
<tr>
<th>power</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.80</td>
<td>290</td>
</tr>
<tr>
<td>0.85</td>
<td>335</td>
</tr>
<tr>
<td>0.90</td>
<td>397</td>
</tr>
</tbody>
</table>

In summary, a sample size of 397 would give a power of 0.90. Given that our n = 475, we believe our sample size is more than adequate. The power calculation has been added to the text in the Results section.

33) In the footnote in Table 2, indicate which statistical test was used to calculate the p-value.

**RESPONSE:** Revised as suggested.

34) Multivariable analysis should be done for all the supplements/nutrients to examine how they are correlated to the outcome. It may be of interest to see whether supplement use is correlated as well.

**RESPONSE:** Multivariate analysis was done for all nutrients (as stated for model 3). Out of interest for space (word) limitations we did not include in the manuscript the Spearman correlations (with Bonferroni corrections) used to examine correlation among the nutrients. Statistically significant correlations found included: the B vitamins (but not folate) were correlated with each other, and weakly (< 0.3) correlated with Omega 3, but negatively correlated with selenium. Vitamin D was weakly correlated with Omega 3 and selenium, and iron was moderately \((r = 0.4)\) correlated with zinc. We also examine the correlation between the nutrients and the outcome of EPDS, and no significant correlations were found. Given that only selenium was statistically significant in our models, after adjusting for all other variables (all nutrients), we believe our findings were robust. Furthermore we mentioned multicollinearity as a possible limitation.

35) The sociodemographic variables should also undergo multivariable analysis and the significant ones should be used in the final model to adjust for in the analysis as confounders. Please revise the statistical analysis of the manuscript as described above.

**RESPONSE:** Multivariate analyses were done with all the sociodemographic variables, and were described in the Methods section: “Using multivariate logistic regression, the first model consisted of all the demographic variables; model two included social/lifestyle variables plus the significant variable from model one. The third model assessed nutrients from supplement intake, and the fourth model incorporated significant (and close to significant) variables from models one, two, and three.”
36. Authors’ information: Please place the Authors’ Contributions section after Competing interests. Please check the instructions for authors on the journal website for the correct format to use for Authors’ Contributions.

**RESPONSE:** Authors’ Contributions added as requested (page 20)

37. Please include a Conclusions section in the manuscript: This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

**RESPONSE:** Conclusion added as requested (page 17).