Author’s response to reviews

Title: Mediating Role of Arsenic in the Relationship between Diet and Pregnancy Outcomes: Prospective Birth Cohort in Bangladesh

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Author’s response to reviews:

Dear Editor:

Enclosed is our revision to the submission, titled “Mediating Role of Arsenic in the Relationship between Diet and Pregnancy Outcomes: Prospective Birth Cohort in Bangladesh” (ENHE-D-18-00138).

We appreciate reviewers’ comments and had revised the manuscript accordingly. Please kindly see the point-by-point response below.

We have uploaded the revised manuscript and supplementary material with tracked changes in the online submission system. We appreciate the Environmental Health for the consideration of the manuscript. Please let us know if we can provide additional information.
Yours sincerely,

Pi-I D. Lin, ScD

Summary of reviewers’ comments:

Reviewer 1 Overall comments: This study contributes to epidemiological research regarding the health effects of prenatal As exposure. Building upon previous research identifying positive associations between GA, GWG, and BW and carbohydrate intake, but negative associations with protein, fat, and fiber, this study investigates As as a mediator. The study has the strengths of a large sample size with biomarker and nutritional data. The manuscript could be improved with clarifications in the results and discussion section. Additional sensitivity analyses may provide information about the contribution of micronutrients, if data are available. Grammatical errors should be corrected throughout the manuscript.

Response:

We thank the reviewer for noting the importance of our study’s contribution to epidemiological research on the health effect of prenatal arsenic exposure. The goal of our study is to build on our previous findings and further explore the causal mechanism of the associations we observed between pregnancy outcome (gestational age, gestational weight gain and birthweight) and macronutrient intakes. We agree that the manuscript needs clarification in the results and discussion, and have made revisions based on the specific comments. We appreciate the suggestion to add a sensitivity analysis on the contribution of micronutrients. However, currently we do not have an appropriate measure of micronutrient intake to perform such analysis. Finally, we have used a professional English editing service (Fresh Eye Editing, LLC, https://www.fresheyesediting.com/) to fix grammatical errors in the manuscript.

Specific comments from Reviewer 1:

Introduction

Lines 109-11: These references (33-36) do not all include analyses of interactions between arsenic and folate status - please confirm references cited here.

Response: We removed the two references that assessed low protein intake but did not examine the interaction with folate status.
Lines 114-116: Are there data on As concentrations in these foods? If so, this information should be included.

Response: We added As concentrations in these foods as suggested by the reviewer.

Methods

Lines 150-151: Please confirm if the water samples were collected postpartum.

Response: Yes, these were collected at one month postpartum, and this information is noted in the methods.

Lines 162-163: Please describe if health care workers were trained in administering FFQs. Please describe if FFQs were designed to ask about specific time intervals (e.g., month 1, month 2, etc.).

Response: All health care workers were trained to administer the FFQ, and participants were asked to recall their diet in the preceding 12 months. We have clarified this in the methods.

Lines 169-172: Include p-values for correlations. It is not clear that these correlations were determined previously in FFQ validation - this would be clearer if this sentence were moved to the beginning of the paragraph

Response: We added P-values for Spearman correlations and clarify the description in the methods for the validation of the FFQ.

Lines 207-208: Was the correlation between maternal education and income assessed? It may not be necessary to include both of these potential confounders in the model. How were education and income categorized? If categorized as listed in Table 1, it may be more appropriate to combine <25 USD and 25-36 USD due to the small N in the <25 USD category.

Response: Income and education were correlated, though not strongly [Spearman correlation, rho=0.25 (p<0.001)]. Due to the unique family structure of our study population in rural Bangladesh, most females were housewives and family income was primarily provided by male heads of households. We believe that pregnant women’s education and household income may have different effects on pregnant women’s diet. Specifically, family income provides a broader
evaluation of the quality of the entire family while a pregnant woman’s education status may shape her own food choices, thus we include both covariates as confounders in the analysis. When assessing the multivariable linear regression model, we did not find an issue of multicollinearity; both education and household income had variance inflation factors <1.5. We applied the reviewer’s suggestion to group income <25 with 25-36 USD group, and revised the table accordingly.

**Results**

Lines 259-260: Table 3 should be referred to in the first sentence of this paragraph. Lines 261-262: Table 4 should be referred to in this sentence.

Response: We have revised the sentence accordingly.

Lines 323: What effect size was used for power calculations?

Response: We used the effect size observed for each of the associations tested to calculate power.

Were any sensitivity analyses performed controlling for micronutrient intake known to influence As methylation capacity and birth outcomes (e.g., folate and other one-carbon metabolism micronutrients)?

Response: We appreciate the reviewer’s suggestion to add a sensitivity analysis on the contribution of micronutrients, another important mediator to evaluate. However, we do not have any micronutrients with proven validity to perform a sensitivity analysis.

Were any sensitivity analyses performed controlling for water As as a confounder? Water As may be associated with both toenail As and birth outcomes, acting as a confounder, rather than an effect modifier (as tested for in stratified analyses).

Response: We did include water As in the model in the initial exploration, and observed a similar direction of effect. Water As is highly correlated with toenail arsenic, and water As concentration was unlikely upstream of maternal diet intake in the causal pathway. Thus, we removed water As concentration from the model as a potential confounder, but examine it as an effect modifier.
Discussion

- The mean energy intake of 3,049 kcal/day seems high particularly for this region (Table 2). Are there data on potential differential bias in FFQ reporting relative to other confounders (e.g., income, education)? The authors should report if this overestimation is biased by As exposure, birth outcomes, or any confounders.

Response: We do recognize this FFQ overestimated the absolute intake value and present this limitation in the discussion. However, we still deem this FFQ as a valid instrument to rank relative intake. In a previous analysis, we found the FFQ to have strong validity in correctly ranking participants’ intake level, and the overestimation was non-differential with participants’ social-demographic status. We revised the discussion to state this.

Lines 358-360: If the FFQ gathered information on the relative consumption of rice and other grains, please report this data. The association between carbohydrate consumption and birth outcomes is puzzling, and data regarding replacement of rice with other grains would help to explain this observation.

Response: In the future, we can use a food composition model to examine the effect by replacing rice consumption with other grain. However, the current mediation model was not adopted to account for multiple exposures. Since the goal of this paper is to assess mediation, we focused on evaluating the mediating effect by As concentration for each individual macronutrient intake.

Were changes in energy and macronutrient intake throughout pregnancy captured by the FFQ, or assumed to be constant? If so, the implications of this assumption should be discussed.

Response: The FFQ captured the dietary habit for the entire pregnancy, so we were not able to evaluate change for specific time windows during pregnancy. We do, however, acknowledge the importance of intake at different time points during pregnancy.

Tables and Figures

- Table 1: Want does the subscript 1 refer to within the table? Please report descriptive statistics for energy and macronutrient intake.

Response: We did not intend to include a subscript 1 for Table 1. We have revised the table to ensure appropriate labeling. We appreciate the reviewer’s suggestion to include energy and nutrient intake, but considering the complexity of the table, we present the descriptive statistics for macronutrient intake in the legend of Table 2. Considering that the absolute intake calculated
by FFQ may be overestimated, we want to focus our analysis on the relative level of intake rather than the absolute amount of intake; thus, we present the birth outcome according to quartile of intakes.

Table 2: Effects significant at 0.05 should be indicated with an asterisk. As written, it is not clear if effects with CIs with lower or upper bounds of 0.0 are significant. Table S1: The number 1 should be superscript in the table legend. Tables S2-7: P<0.05 should be indicated. Figures S1-3: The legend should specify that the numbers in parentheses are SEs.

Response: We made revisions as recommended by the reviewer.

Reviewer 2’s Comments:

1. The manuscript seems lengthy; it should be more concise.

Response: We appreciate the reviewer’s comments to make the manuscript more concise. We shortened the background and methods sections by removing information that can be found in previous publications, and revised results and discussion to focus on the most important findings.

2. The authors should prove that their model (mediation model) is better than an ordinary analysis such as the multiple regression analysis. Also, can the energy, protein, fat, carbohydrate and/or fiber in diet explain the source of arsenic exposure in subjects? The unit of variables (per SD increment) is perplexed to grasp the effect size.

Response: We revised the background and discussion to highlight the advantage of using the mediation model compared to multiple linear regression analysis according to the reviewer’s suggestion. Our analysis did show energy, protein, fat, carbohydrate and fiber to be associated with toenail As concentration, and our previous studies (mentioned in the introduction and discussion) also found that a high proportion of As exposure in our study population came from dietary intake. The way we present our analysis on nutrient intake is consistent with the convention used in other nutrition epidemiology studies. In addition, since the FFQ was limited in its accuracy to measure absolute intake, we were not comfortable in using other absolute unit increments (e.g., per 10 g). We believe presenting our effect estimate per standard deviation increase is a more conservative way to compare the relative intake levels among our study population.
3. The GA and GWG were significantly affected by arsenic exposure. The authors should stress this positive observation rather than the negative result on BW, which might have been due to less reliability of BW measurements in newborns.

Response: We agree with the reviewer and took out the discussion on the negative result on BW in the discussion and focus the discussion on the positive observations.

4. The toenail arsenic is more reliable than other exposure indices such as blood levels? Evidence should be given.

Response: Toenail As concentration is a reliable biomarker of long-term As exposure. We revised the methods section according to the reviewer’s suggestions and included evidence by citing previous publications.