Author's response to reviews

Title: Biomonitoring of Bisphenol A Level in Maternal and Umbilical Cord Blood Relevant to Birth Outcomes and Adipokine Expression

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Author's response to reviews: see over
Response to Reviewers’ Comments for MS-1312412857516153

Thanks for reviews’ valuable comments. These comments greatly improved the quality of this manuscript. The manuscript has been revised with the comments carefully considered. The following are the detailed responses to each individual comment. We use the yellow markings to highlight the changes and new entries in the text. Additionally, we also double check our manuscript and revise properly.

Reviewer #1:
Major comments:

1. This manuscript’s major weakness is its lack of clarity and large amount of textual errors. The study’s findings are interesting, timely, and contribute to the field but the manuscript needs to be substantially reworked. Moreover, it is unclear how the statistical analyses were performed.

Response: Thanks for your suggestions. We have carefully reworked this manuscript based on the comments. Moreover, we revise the description of statistical analysis in page 9, “Statistical analysis”.

2. Neither in the title nor manuscript mentions the type of study design. Although the authors describe the design in the methods section, it is not explicitly named with a commonly used term per the STROBE guidelines.

Response: We consulted the STROBE guidelines to revise the text.

3. In the methods section, there is no suggestion of how the study size was determined nor the recruitment time frame. When during pregnancy was maternal blood collected for the BPA assessment? It is also unclear why the exposure variable (BPA) is modeled with binary “high and low” levels in Table 1 and then subsequently in tertiles (though incorrectly named quartiles). For the binary “high and low” modeling, the manuscript does not define the cut point, though this information is listed in Table 1. Furthermore, it remains unclear whether reference is made to the maternal or the umbilical cord blood BPA in the table legends.

Response: We have described in detail about when maternal blood was collected during pregnancy in pages 6 and 7, line 121-132. Because of previous studies weren’t defined the cut points of the adverse outcome posed by BPA, thus this study would like to explore the effect of fetal outcome underlying BPA levels, and elaborate the dose-response relationship. The study subjects were divided into two groups based on the geometric mean of maternal BPA levels.
4. The coefficients of variation for BPA assessments are not provided. There is not mention of any laboratory quality control measures for any of the analytes.

Response: Thanks for reviewer’s comment. We described QA/QC of laboratory analysis, and non-detects handled in the page 7-8, line 153-159.

5. Why was low birthweight defined as <2600g? Similarly, the cut-off levels for high and low adipokines are not provided.

Response: We addressed the description of cut-off levels for LBW, high and low adipokines in page 8, line 174-181.

6. In regards to statistical methods, the authors do not address the manner in which they handle missing covariate data, if there is such missingness, nor the way in which they model BPA levels below the limit of detection (LOD).

Response: We described how to handle missing covariate data for statistical analysis in page 7, line 155-157 “Concentrations below the limit of detection (LOD) of 0.13 ng/ml were given a value of LOD/2 for the statistical analyses”.

7. When the authors present the unadjusted and adjusted analysis in the results section, there is no explanation about why each of the potential confounders is being included. Although it is mentioned in the methods section that the confounder data is collected via self-report patient questionnaire, there is no description for how these variables are modeled. We can assume all variables are modeled continuously based on their presentation in Table 1 but this could be stated more clearly. The text, table, and figures report only odds ratios but the authors make numerous references to risks ratios, which may have been a typographical error or a larger methodological issue.

Response: Thanks for your comments. We have added the explanation about the potential confounders in page 9, line 184-190.

8. Table 2: Most of these correlations are not informative. Suggest deleting. Just keep correlations between maternal and fetal BPA levels in the text.

Response: Thanks. We have revised Table 2 according to your suggestions to delete some items.
Table 3: It is unclear how this association was modeled. What was the dependent variable? What was the independent variable? Was the independent variable binary or continuous or differently categorized? No tests for heterogeneity by gender were performed.

Response: In Table 3, the dependent variable is fetal birth outcome, and the independent variable is binary maternal BPA level. We added the description in page 9, line 184-190.

9. Figures 2, 2A and 2B mentioned in the text are missing.
Response: Sorry for the confused. We have revised to mention for Figure 2A and figure 2B in the text.

10. Lastly, in the discussion section, there is a nice interpretation of the results contextualized in regards to similar studies. However, the Furthermore, there was not discussion of the study’s limitations nor the generalizability.
Response: Thanks, we have added the study limitation in Pages 16-17, line 380-397.

Minor comments:
Response: We have carefully revised this manuscript based on your suggestions. The changes and new entries in the text are highlight used the yellow markings.
Reviewer #2:
General Comments:
1. Background: In the review of the animal literature the authors need to clearly identify dosing routes since oral routes are more likely to be relevant to human internal doses. Also, more care need to be taken in describing internal doses; what is the evidence that BPA ‘accumulates’ in adipose tissue? Response: Thanks for your suggestions. We have clearly described dosing routes in the page 4, line 76-77, line 80-81 and line 84-85. Moreover, we added the description about BPA levels accumulated in adipose tissue in the page 5, line 91-93.

2. Recruitment and sampling methods need to be better described. When was the study conducted? Was there IRB approval? How were subjects were identified and recruited? How were women approached and what was the response rate? What information was collected on the questionnaire and what steps were taken to make sure that the questionnaire was properly administered? At what point during pregnancy were maternal samples collected? How were data including blood samples collected? Were precautions used to assure that sample containers were not contaminated with BPA? Response: We have added the descriptions of study duration, IRB approval, subject recruitment, sample collection, and questionnaire response and information at page 6 study subjects and page 7 sample preparation.

3. Laboratory methods need to be better described. Measure of low levels of BPA in serum can be confounded by background levels of BPA in laboratories and in laboratory equipment. The paper needs to describe the QA/QC procedures used by the laboratory. The situation with regards to non-detects. How many of the results were non-detects? How were non-detects handled in data analysis? Response: The descriptions of QA/QC procedures and non-detects handled in data analysis were in the page 7-8, line 153-158.

4. Statistical methods: What was the approach to modeling and selection of control variables for multiple regression analyses? Did the investigators assess potential effects, effect-modification and/or confounding by factors known to impact LBW and SGA, most notably maternal smoking, parity and socioeconomic status?
Response: Thanks for reviewer’s comment. We have revised the footnotes in Table 3 and explained the confounding factors in each model. We also described the approach to select control variable for logistic regression model in **Page 9, line 185-190.** In analysis to estimate whether maternal smoking and socioeconomic status impact LBW and SGA, the results of Chi-square test did not show any significant differences in LBW (smoking: p-value=0.982; socioeconomic status: p-value=0.914) and in SGA (smoking: p-value=0.774; socioeconomic status: p-value=0.771).

5. Results: The paper reports that there is a relationship between BPA exposure and all four outcomes in male infants. These findings could have been significantly influenced by just a few infants and it is important to make sure that such results aren’t driven by just a few influential points. Two of the outcomes (LBW and leptin) show elevated odds ratios for both the 2nd and 4th but not 3rd quartiles of BPA whereas SGA and adiponectin show an elevated OR in the 4th quartile only. The former relationships are interpreted as “u-shaped” dose response curves in the discussion. However, the lowest dose level is not a “zero” or control level like in animal studies but actually is the “low dose” level. If there were a u-shaped dose response the response would be higher at these lower doses than at intermediate dose levels. These dose response curves more resemble a seesaw relationship than a u-shaped curve. In any case the data for females was interpreted inconsistently. All of the results for females were interpreted as null findings however the pattern of odds ratios for SGA is similar to the pattern among males for LBW which are interpreted significant relationships.

Response: Thanks for your suggestions. The biological effect of BPA in human body is complicated, and until now no study can definitely defined the threshold for the dose-response relationship. Therefore, this study used the “low dose” level of BPA as reference to calculate odds ratios (**Figure A**). However, it is surprising to find the result in a “U-shaped” curve. Thus, the relationship between BPA level and effect may be like **Figure B**.

![Figure A](image1.png)  ![Figure B](image2.png)
6. Results: If the BPA blood analyses are valid the ratio between maternal and fetal levels may be the most important finding. However, it is unsettling that, as stated in the discussion, there “was no significant correlation” between maternal and fetal levels. Were the samples taken at disparate points in time? Also, discussion cites a study reporting that 27% of BPA crosses the placenta. If so, why are the levels in this study so much lower? This needs to be discussed.

**Response:** Our study found that 13% transfer percentage of BPA levels from maternal blood to fetal cord blood. The difference from previous studies may be due to the individual difference or system variations because they are *in vitro* experiment with a small sample size (n = 7). Furthermore, we discussed further in page 13, line 288-292 and page 16, line 366-379.

7. Discussion: Please discuss the findings in the light of the fact that there is a very short biological half-life of serum BPA the issue of timing of sample collection and relevance of a single exposure level at a single point in time to longer term outcomes.

**Response:** Thanks for your comments. We had added the discussion in pages 16-17, line 366-380.

8. If information on smoking, socioeconomic status and parity was not available this is a major limitation of this study and needs to be discussed.

**Response:** We discussed the limitation in page 17, line 380-389.

**Minor Essential Revisions:**

1. Please describe how were the “adverse action” cut points for adiponectin and leptin determined?

**Response:** We described the “adverse action” cut points for LBW, SGA, adiponectin and leptin in page 8, line 174-181.

2. Results: Table 3 needs to include the numbers of infant/mother pairs included in each of the 4 groups for the outcomes in the multiple regression analysis.

**Response:** In Table 3, we revised to include the numbers of infant/mother pairs.

3. Discussion: The discussion goes into numerous issues (e.g. breast development and brain differentiation) that are not directly related to the study hypothesis and study findings. The discussion needs to be focused on
fetal growth and metabolism.

**Response:** Thanks for reviewer’s suggestion. We have revised the description in page 12, line 275-281.