Author’s response to reviews

Title: Exploring telomere length in mother-newborn pairs in relation to exposure to multiple toxic metals and potential modifying effects of nutritional factors

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telomere length in mother-newborn pairs in relation to exposure to multiple toxic metals

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Point-by-point response to comments by editors and reviewers:

Regarding a point raised by our Editorial Adviser 1, we agree that it would be appropriate to re-introduce the words ‘Nutritional factors’ in the title, and to ensure the results and conclusions state that nutritional factors were looked into, but no significant results were found.

Response: We have included “nutritional factors” in the title, which now runs: “Telomere length in mother-newborn pairs in relation to exposure to multiple toxic metals and potential modifying effects of nutritional factors.” Also, we have added this to the aim in the abstract: “Objectives: To assess the potential impact of multiple toxic metals on relative telomere length (rTL) in maternal blood, cord blood, and placenta, as well as potential modifying effects of pro-oxidants.” In the end of the abstract, we had already included: “The studied antioxidants did not modify the associations, except that with antimony. CONCLUSIONS: Elevated exposure to boron, lithium, arsenic, and antimony with maternal or newborn rTL in a tissue-specific, for lead also sex-specific, manner. Nutritional antioxidants did not generally influence the associations.”
In the discussion we comment on the potential modification by anti-oxidants for each main finding. And in the Conclusions, we state: “Nutritional antioxidants did not generally influence the associations.”

Reviewer #3: The paper was substantially improved and the authors addressed the main concern regarding the first round of revision.

Response: We are very pleased with this comments and would like to repeat our appreciation of the constructive comments by the reviewers concerning the focus of the study. Indeed, the manuscript has improved substantially.

Editorial Adviser 1: I would advise that the overall study aims are not changed, as this would be misleading. I would want to see a statement of what the false discovery rate was that the authors had set, a clear statement that the authors had carefully set this false discovery rate in advance, and that they provide suitable justification for their choice. This should include all micronutrients and subgroup analyses too.

Response: In line with previous comments from the reviewers, we have clarified the aim and strengthened the focus of the manuscript. We have not changed the overall aim, but clarified that the aim is based on the hypothesis that toxic metals with pro-oxidant properties may affect the telomere length. Since the first submission of this manuscript, the hypothesis has strengthened further, why we have expanded the background information about the effects of Cd on TL somewhat. Please, see further comments above by reviewer #3, who previously expressed strong recommendations to focus the manuscript. See also the response above to the editor concerning re-introducing nutritional factors in the title and the conclusions.

We have tried to better explain the use of the false discovery rate in the methods section (pages 9-10, lines 226-232) as follows “We used the false discovery rate (FDR) of multiple testing correction according to the Benjamini–Hochberg FDR method (34) and applied a significance threshold of q < 0.05. Basically, the obtained lowest p value was multiplied by the number of tests performed for each media (maternal blood, placenta and umbilical cord blood), the next lowest by the number of analyses minus one, and so on. The same process was applied for the evaluation of the micronutrients evaluated as potential modifiers.”

We are aware of the problem with multiple testing, but also with the increasing concern that correction for multiple testing may increase the type II error (see e.g. Rothman, 1990). Also, many journals are less keen on strict focus on p-values, but rather recommend stressing the effect size and its precision. We do provide confidence intervals both before and after adjustment. And, we carefully evaluate and discuss each finding against previously reported data and the effect size (see further the subsequent point).

Editorial Adviser 2: I am not sure if the beta coefficients they are reporting in Table 3 have much meaning. They don't really discuss what the values mean. Since they apply to different exposures that may be measured in different numerical levels, the numbers may not be comparable across
exposures. It would be nice if they included some kind of explanation as to what the units are for the regressions. Presumably they are increases in rTL per 1 mugram/Liter increase in the exposure. They should mention that in the text and/or footnote to the table. I don't know if a microgram/L of cadmium is comparable to a microgram/Liter of cesium for example.

Response: The B coefficients are not the change in rTL per µg/L (or µg/kg) of the metal concentrations, for the reasons mentioned. We previously wrote in the Methods section that we log-transformed (log2) all metal concentrations as most showed highly skewed distributions and the transformed data produced better fits (page 10, lines 232-234). Using log2-transformed concentrations implies that the estimates represent the mean change in the outcome for a doubling of the exposure. Thus, the effects size can be compared between the different media and between metals. For clarification, we added the following sentence in Methods, page 10, lines 234-237: “We chose log2-transformation of the metal concentrations because it provides a simple interpretation of the B-coefficient in the linear regression analysis, i.e., the mean change in outcome associated with doubling of the concentration.” In addition, we made clear in the Abstract that we used log2-transformed metal concentrations (page 2, lines 40-41), in the Result sections, as well as in the heading of Table 3 (page 28).