Author's response to reviews

Title: Lead, mercury and cadmium in umbilical cord blood and its association with maternal epidemiological variables and birth factors.

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Author's response to reviews: see over
REVIEWER 1

We would like to thank the reviewer for helping us improve the new version of the manuscript.

Major compulsory concerns:

1. Page 11: Authors reports that “no paternal factors were associated with cord blood metal measurements. However, the authors never report what paternal factors were assessed.

We completely agree with the reviewer, and since Reviewer 1 also indicated the importance of including this information in the manuscript, we have now added an extra table (table 3). This table contains lead, mercury and cadmium levels by paternal characteristics, while shows geometric mean ratios across defined paternal categories. The results of this table are now discussed in the manuscript.

2. Page 12: The CDC level of concern is 5 ug/dL or 0.5 ug/L (not 5 ug/L). Authors need to confirm the units that were used in their analyses.

The reviewer is right; this was a typo and has been corrected. Also, the units have been reviewed along the text.

3. Page 15: The authors recommend that “mothers to quit smoking and reduce mercury intake by choosing low-mercury fish during pregnancy.” However, this is already the recommendation for pregnant women. This study doesn’t appear to provide any new novel findings.

We agree with the reviewer that these are already general recommendations for pregnant women. However and despite the evidence, many women keep smoking during pregnancy. In Spain, around 25-35% of pregnant women smoke during pregnancy (Aurrekoetxea et al., 2013; Bolumar et al., 1994; Jimenez-Muro et al., 2012; Puig et al., 2012). Although this prevalence was lower in our study (16%), still we think it is important to highlight this recommendation in our setting.

Regarding fish intake, in Spain recommendations against consumption of high-mercury species during pregnancy are relatively recent. In 2008, the Spanish Food Safety and Nutrition Agency published, for the first time, recommendations regarding fish consumption during pregnancy or early childhood (for additional information regarding this issue please see http://www.aesan.msc.es/AESAN/web/cadena_alimentaria/subdetalle/qui_mercurio.shtml.)

Our results suggest that maternal fish consumption patterns are determining the high umbilical cord blood mercury levels observed in BioMadrid (with more than 70% exceeding the safe level established by the EPA). Accordingly, we think it might be desirable to insist on this point. The revised version of the manuscript tries to better address this issue as follows:

“Because of the potential deleterious effects of metal exposure on newborns health, more efforts should be made to recommend mothers to quit smoking and reduce mercury intake by choosing low-mercury fish during pregnancy”.

Finally, we would want to highlight here the fact that although many countries have now well established biomonitoring programs, in Spain there are very few studies on the distribution of environmental pollutants in the general population or in high risk groups. Also, regardless the limitations of the study, we provide some new evidence that relatively low cadmium concentrations may be deleterious to fetal health.
Minor essential concerns:

1. Page 4, first paragraph: Additional effects to pregnant women can occur from lead exposure. These are reviewed in:
   We thank the reviewer for providing us with this interesting document. We have now completed the paragraph as follows
   “Lead exposure can also cause spontaneous abortions (Borja-Aburto et al., 1999), congenital malformations (Irgens et al., 1998; Needleman et al., 1984), reduced birth weight (Bellinger, 2005) or length (Hernandez-Avila et al., 2002), gestational hypertension (Kennedy et al., 2012), pre-eclampsia (Kennedy et al., 2012) and impaired neurodevelopment (Bellinger et al., 1986).”

2. Page 4, first paragraph: Need to define “MT expression”.
   We have changed MT for metallothionein.

3. Page 4, 2nd paragraph: Other important lead sources are lead-based paint that may be peeling from walls, and lead-based gun ammunition (i.e. bullets).
   The reviewer is right that there are other sources of lead exposure. Particularly regarding children, lead-based paint remains an important source of lead exposure. We have added this information to the text as follows:
   “Sources of lead include leaded gasoline, lead paint hazards (including lead in paint, dust and soil), water carried out in lead pipes, industrial emissions or occupational exposures (Tong et al., 2000).”

4. Page 5: Need to better specify the “complications in logistic procedures in the remaining 5 cases”.
   This part of the Methods section has been changed for better comprehension. The text now reads (third paragraph, Methods section):
   “Logistical problems (births at non-participant hospitals due to overcrowded maternal wards in the reference centers, mothers who forgot the kit, problems to draw blood from the umbilical cord vein and samples stored incorrectly), precluded the availability of cord blood in 27 participants, while in 4 cases problems at delivery did not allow obtaining the samples (one child was born dead and in three cases an emergency cesarean section was needed)”

5. Page 6: Should “logistic reasons” be “logistical reasons.””? 
   This was a typo. We have changed logistic for logistical.

6. Page 8: Need to specify the number of reports with Cd levels were below the LOD.
   We agree with the reviewer. We have corrected the text as follows:
   “For cadmium, 47% of samples in cord blood and 15% of samples in peripheral blood had concentrations below 0.25 µg/L”.

7. Page 8 and 9: Change “study level” to “education level”.
   This has already been changed
This has been changed here, as well as in table 2.

9. Page 10: The mean weight needs units. Is it 3282 grams?
The reviewer is right. The word “gram” has been added to the text.

10. Page 14: It’s not clear what is meant by “there is only more study evaluating...”
This was a typo and should have read “there is only one more study”. However, since we first sent
the manuscript to review, a new study by Al-Saleh and colleges has been published, showing a
consistent negative association between cadmium levels in umbilical cord blood and the Apgar
score. Accordingly, we have changed this section of the discussion.
REVIEWER 2
We would like to thank the reviewer for helping us improve the new version of the manuscript.

1. Discretionary Revisions

There are some words that I would propose to change in this paper; 1) Study level # Education level, 2) Race # Ethnicity
As proposed by the reviewer we have changed study level for education level, and race for ethnicity.

2. Minor Essential Revisions

All suggested changes in this section have been applied

-Please make sure that there is always a space before and after the references.
Checked and corrected

-A space is always necessary after a period.
Checked and corrected

-The author wrote the word birthweight and birth weight.
In line with the reviewer's comment, this has been changed to ensure consistency of use.

-P. 7, there is two A at the word Analyst
Checked and corrected

-P. 8, please number the statistical analysis section.
Done

-P. 10, second paragraph, should add gr after 3282.
Done

-P. 11, last paragraph, Doi et al, 1984. Please review all the references along the text. The year of publication is missing for some references.
All the references have now been checked.

-P. 13, third paragraph please put a comma after the words In Spain.
The comma has been added

-P. 14, second paragraph, I don’t understand the term “only more study”.
There was a typo in this section. It should have read “there is only one more study”. However, since we first sent the manuscript to review, a new article evaluating the association between cord blood cadmium and the Apgar score has been published. In this report, based on 1578 newborns with a mean cadmium concentration of 0.78 µg/L, Al-Saleh and colleges show that newborns with Apgar 5-minutes scores below the 10th percentile (0-8) have higher levels of umbilical cord blood cadmium (Al-Saleh et al., 2013). According to this new finding, the sentence now reads:

“To our knowledge only two studies have previously evaluated the association between cord blood cadmium concentrations and Apgar scores (Al-Saleh et al., 2013; Mokhtar et al., 2002).”

-Table 1, Fish intake, please add g/day for the category 60-100 g/day.
Done

-Table 1, there are p-value <0.05 with asterix and some not.
Corrected.

-Table 2, the word cadmium has two “m”.
Corrected.

-Table 2, in the footnote, please put a period after the parentheses.
Corrected.
3. Introduction section:

Please add references for the statement: “Occupational exposure to mercury has been associated with pregnancy-induced hypertension, low birthweight and birth defects.

The corresponding references have been added to the text:

“Occupational exposure to mercury has been associated with pregnancy-induced hypertension (Pan et al., 2007), low birth weight (Ramon et al., 2009) and birth defects (Jin et al., 2013)”.

4. Material and methods section:

-P. 6-7, because birth weight and birth length are used as outcomes in the statistical analysis, the description of the methods for measuring them should be specifically included in the paper. Please mention if several measurements were done for each anthropometric parameter and if so, how these measurements were handled in the statistical analysis?

For each anthropometric parameter, only one measure was obtained. We have now included an explanation on how measures were taken (fourth paragraph, methods section):

“During hospitalization, a protocolled clinical examination of the newborns was performed to collect data on sex, gestational week and anthropometric birth factors and possible malformations. Anthropometric data were measured once, before breastfeeding started. Infants were weighed without diapers and using an electronic digital infant scale. Length was measured in the supine position, using a stadiometer composed of a stationary head-board and a movable footboard. Knees and hips were extended using gentle force and the footboard pressed against the balls of the feet.”

Since we agree with the reviewer that this is an important point, we have now included this issue in the discussion section, among the limitations of the study.

“Also, anthropometric measurements were performed only one time, resulting in a potential source of measurement error. However, the complexity of the field work, carried out within the public health system, did not allow for collection of additional measurements”

-P. 7, Instead of saying that the sample size was 318 participants (106 mothers, 106 fathers...), the author should state that the sample size was 106 complete trios. It’s the trio that is important, not the sum of the newborns, mothers and fathers

Following the reviewer’s suggestion, we have changed this section:

“In this study we first described metal levels in all BioMadrid participants with blood samples available (140 mothers, 140 fathers and 114 children). We then excluded trios with no cord-blood samples available or with problems at delivery, trios where at least one member had no metal determinations, and trios where at least one adult had not completed the epidemiological questionnaire, leading to a final sample of 112 complete trios. Finally, in the sub-sample of trios with all information available, we evaluated the possible associations between parental factors, umbilical cord blood metal levels and newborn’s characteristics at birth.”
5. Results section:

-P. 10, first paragraph. The mean age of 32 years, is for the mothers, the fathers or both. The mean age should be presented separately for mothers and fathers. We agree with the reviewer. Now, the sentence reads as follows:

“The mean age was 31.1 years for mothers and 32.0 for fathers.”

-P. 10 third paragraph. The author state that 15% of the samples were under the limit of detection for cadmium, while on p.7 she said that 47% of cadmium samples were below the limit of detection.

The reviewer is completely right. In 47% of cord blood samples and 15% of peripheral blood samples cadmium was below 0.25 µg/L. This has been changed in the methods section:

“For cadmium, 47% of samples in cord blood and 15% of samples in peripheral blood had concentrations below 0.25 µg/L” (Second paragraph, section 2.2).

In order not to be repetitive, this sentence has been removed from the result’s section in the revised manuscript.

-P.10, fourth line of third paragraph. How could geometric means for cadmium in cord blood range from 0.23 to 0.31 ug/L if the detection limit is 0.25 ug/L? Also in Appendix A, the p25 is below the detection limit for cord blood and fathers. The author should indicate <LD instead of a concentration. Was the author replaces the concentration of cadmium below the limit of detection by half the detection limit for the analysis as mentioned in the statistical analysis section? The presentation of GMs with levels below the detection limit gives the impression that this was not the case

We regret that this proved quite so confusing. As stated in the statistical analysis section, several methods for treating values below the limit of detection (LOD) have been proposed in the literature. In our study, we applied the method proposed by Hewett and Ganser, whereby values under the LOD are replaced by half their detection limit. This means that values under the LOD for cadmium (0.25) are treated as if their real value were 0.125 (LOD/2). On the “statistical analysis” section the first sentence has been modified as follows to make it easier to understand this:

“Cadmium values below the detection limit were replaced by half the detection limit for statistical analysis (Hewett and Ganser, 2007).”

6 Tables

Usually geometric means are not presented if more than 40% of samples are below the detection limit. This is the case for cadmium according to what is written on page 7

We agree with the reviewer that this is the case in the case of cord blood determinations. However, for consistency with the results obtained in adults (with only 15% of cadmium concentration below the detection limit), we would prefer to maintain geometric means as presented.

Major Compulsory Revisions

7. I have one major concern with regard to this paper. The authors never explain how was made the selection of potential confounders. It’s seems that the choice of adjusting for specific variables in multivariate models was arbitrary. For example, in the model for birth weight (Table 2) why cigarette smoking was included in the model for mercury and cadmium, but not lead, as we know that
maternal cigarette consumption is a risk factor for low birth weight. Also, the authors never discuss potential bias in their results that could be related to the absence of adjustment for important variables (maternal alcohol consumption, socioeconomic status, obstetrical history, ...).

We regret that this was not more explicitly stated in the previous version of the manuscript. We have now attempted to better address this issue by explaining the adjusting variables that were included in each multivariate model. Briefly, multivariate regression models in tables 2 and 3 were adjusted for well-known risk factors that have been described in the literature as main determinants of mercury, lead or cadmium concentrations (see McKelvey et al, 2007). In this sense, these models adjusted for parental socio-demographic characteristics (age, living region and educational level) and for well-known “specific-related factors” (fish consumption in the case of mercury and cigarette smoking in the case of cadmium). Additionally, because cord-blood lead concentrations in our study were associated with “sampling season” and with “maternal tobacco smoke”, we further adjusted lead models for these two variables, but because results did not change these “further adjusted models” are not presented in the tables.

Regarding table 4, we have also followed the strategy of analysis reported in previous studies. In this regard, models were adjusted for maternal age, newborn’s sex and gestational age (since these variables are known to influence birth outcomes), and for those potential confounders associated with metal concentrations in our population (maternal tobacco smoke and trimester of the year for lead; fish consumption for mercury and maternal tobacco smoking for cadmium). This is now also explained in the statistical section.

Table 2 now contains additional information regarding the association between mercury, lead and cadmium concentrations and certain maternal variables (including those related to maternal obstetric history). Because none of these factors were associated with cord blood metal levels, this information was initially excluded from the table to avoid it being too long. In regard to alcohol consumption, this is not shown in the table because “only” 3 mothers reported drinking alcohol during pregnancy, and their alcohol consumption was low (<100 gr/week)

The reviewer is right that although we have adjusted for the previously cited variables there is always the possibility of residual confounding. For example, although we have tried to control for socioeconomic status by including parental education in the models, we are aware that this is a complex construct based on different components that include not only education, but income or lifestyles, so the potential of residual confounding is present. To address this issue, we have added the following sentence to the “limitations” section:

“Finally, there is the possibility of residual confounding by factors not completely controlled for by the variables included in the analyses.”

8. Concerning the discussion section, a lot of statements should be referenced. I found that the literature reviews was minimal. There are a lot of studies that were published on the potential effects of heavy metals on birth outcomes, but they were poorly discussed in this paper.

The reviewer is right. We have significantly changed the discussion (please see manuscript).

9. The authors report an association between cadmium and Apgar score, but did not propose a biological explanation (biological plausibility) for this association. They mentioned that Mokhtar et al., had also evaluate the association between
cadmium and Apgar, but we do not know what was the finding.

Again, the reviewer is right. We have also changed this (please see manuscript).

10. Finally, the originality of this paper lies in the fact that they measured heavy metal concentrations in fathers. A lot of studies have documented the relation between maternal and cord concentrations of contaminants, but few with fathers. This should be discussed, even if there are no statistically significant associations. This finding is worth to be discussed.

We agree that including fathers is one of the main strengths of the study. To insist on this specific point, we have now added a table (table 3) that contains information on the association between lead, mercury and cadmium levels and paternal epidemiological characteristics. These results are discussed in the revised version of the manuscript.
Bibliography


