Author's response to reviews

Title: Maternal blood manganese level and birth weight: a MOCEH birth cohort study

Authors:

Jin-Hee Eum (hhsmvp@skku.edu)
Hae-Kwan Cheong (hkcheong@skku.edu)
Eun-Hee Ha (eunheeha@ewha.ac.kr)
Mina Ha (minaha@dku.edu)
Yangho Kim (yanghokm@nuri.net)
Yun-Chul Hong (ychong1@snu.ac.kr)
Hyesook Park (hpark@ewha.ac.kr)
Nam-Soo Chang (nschang@ewha.ac.kr)

Version: 4
Date: 1 February 2014

Author's response to reviews: see over
Dear reviewers,

We appreciate your comprehensive comments and suggestions. We find it is very helpful in upgrading our manuscript. Please refer to our responses listed below.

**Reviewer 1**

**Major compulsory revisions**

**Abstract**

1. The definition of study subjects is not completely reported: only term live born singletons without « severe complications » were selected.

   + As you commented, we add the definition of study subjects as follows:

   We performed analysis on a cohort of 331 mother-infant pairs enrolled in the Mother and Children’s Environmental Health (MOCEH) study in Korea.  

   ↓

   **P2 L8**: We performed analysis on a cohort of 331 *full-term, live birth singleton* mother-infant pairs enrolled *from July 2007 to December 2009* in the Mother and Children’s Environmental Health (MOCEH) study in Korea.

2. The wording of the conclusions should be softened: the « adverse effect » of Mn is not demonstrated, and does not « represent the essential role of Mn… ».

   + As you commented, we revised conclusions as follows:

   The findings of our study show *an adverse effect* of maternal blood Mn level at both lower and higher ranges, representing the essential role of Mn in early stages of human development.

   ↓

   **P2 L22**: These findings suggest that the maternal blood Mn level at both lower and higher ranges are related with adverse birth outcome, reflecting the essential role of Mn in early stages of human development.
Introduction

3. 3rd § lines 5-7: The two previous publications reported as having limited value because «conducted in a population with a high level of environmental exposure… » report in fact mean blood levels very comparable to the present study: 19 μg/L in Vigeh et al., 24 μg/L in Zota et al. versus 22.5 μg/L in the present study.

+ As you commented, we modified introduction as follows:

<table>
<thead>
<tr>
<th>Boxed text</th>
</tr>
</thead>
<tbody>
<tr>
<td>One of the reported maternal and developmental toxicities in studies of experimental animals with high Mn exposure is reduced foetal body weight [11,12]. Maternal blood Mn concentration was associated with adverse birth outcomes [13] and birth weight was biphasically associated with maternal blood Mn concentration in an inverted U-shaped dose-response relationship [14]. However, these studies were conducted in a population with a high level of environmental exposure, limiting analysis of the potential relationship between maternal blood Mn and foetal development [15]. There are few reports on the birth outcome of Mn exposure during pregnancy in the general population. Also, effect of the very low level of Mn was less explored.</td>
</tr>
</tbody>
</table>

↓

P5 L16: Manganese-related maternal and developmental toxicities have been observed in studies of experimental animals, including reduced foetal body weight and high Mn level [12,13]. Few epidemiologic studies reported the relationship between maternal blood Mn level and birth weight of pregnancy outcome in human [14,15]. In a study in Teheran, Iran, intrauterine growth retardation was linearly associated with lower maternal blood Mn level, but with higher cord blood Mn level [14]. In another study, birth weight was biphasically associated with maternal blood Mn concentration in an inverted U-shaped dose-response relationship [15]. This study was conducted in a population living near a lead and zinc mining site in northeastern Oklahoma, U.S.A., with a potential environmental metal exposure. There are few reports on the birth outcome of Mn level during pregnancy in the general population [16]. Also, the effect of the very low level of Mn was less explored. Little is known about the effects of deficiency or excess of Mn on infant growth or birth outcome in humans [16]. Uncertainty still remains about the degree which Mn level will be adequate for pregnant woman.

Methods

4. Whereas the MOCEH cohort includes 953 mother-infant pairs, only 352 were included in the present analysis. The reasons for exclusion (and corresponding numbers) should be given and the representatively discussed.

+ As described in the response to comment 1, manganese study was approved on all the recruited pregnant women in a limited time period, we could study on 352 pregnant women
who were consecutively recruited between July 2007 and December 2009.

Study subjects were 352 mother-infant pairs who agreed to undergo maternal blood Mn analysis and had available birth records among 953 pregnant women and infants who were recruited from May 2006 to January 2008 from three centres.


5. Birth weight has been chosen as marker of fetal growth whereas other studies have also studied birth length, ponderal index or intrauterine growth restriction. Inclusion of these outcomes in the present analysis would widen the evaluation of potential consequences of Mn exposure.

+ We agree with the comment and actually, we have conducted analyses on the birth length and Ponderal index. However, these results were not significant for Mn as shown below. Thus, we included only birth weight in the paper.

- Result of generalised additive model (GAM) analyses for maternal blood Mn and birth length and Ponderal index:
+Result of simple regression model with Ponderal index:

<table>
<thead>
<tr>
<th>Model 1‡</th>
<th>Beta</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Mn</td>
<td>0.00</td>
<td>0.000</td>
<td>0.545</td>
</tr>
<tr>
<td>Model p value</td>
<td></td>
<td></td>
<td>0.545</td>
</tr>
<tr>
<td>Adjusted R²</td>
<td></td>
<td></td>
<td>-0.002</td>
</tr>
</tbody>
</table>

+Result of simple regression model with birth length:

<table>
<thead>
<tr>
<th>Model 1‡</th>
<th>Beta</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Mn</td>
<td>0.00</td>
<td>0.010</td>
<td>0.774</td>
</tr>
<tr>
<td>Model p value</td>
<td></td>
<td></td>
<td>0.774</td>
</tr>
<tr>
<td>Adjusted R²</td>
<td></td>
<td></td>
<td>-0.003</td>
</tr>
</tbody>
</table>
6. Low birth weight is not defined

+ We agree your comment, and we deleted “low birth weight” and replaced that with “birth weight below 3000 g” (P9 L12-13).

7. Maternal diseases during pregnancy should be included among potential confounders

+ We have surveyed following list maternal diseases during pregnancy on all the participants to MOCEH study. However, among 352 participants, following participants were excluded for stillbirth (N=1), pregnancy-related diabetes (N=3), severe fetal stress (N=4), twins (N=4), and premature birth (N=3).

8. It is not clear whether Mn analysis was performed on whole blood, plasma or serum.

+ As you commented, we added “whole blood” in abstract and manuscript as follows:

ABSTRACT
A questionnaire on general characteristics, a review of medical records, and blood Mn analysis were performed at birth of pregnancy.

Blood collection
Blood Mn level was measured by graphite furnace atomic absorption spectrophotometry (AAAnalyst 600, Perkin Elmer, Waltham, MA, USA).

↓

P2 L11: ABSTRACT
A questionnaire on general characteristics, a review of medical records, and maternal whole blood Mn analysis were performed at birth of pregnancy.

P7 L16: Blood collection
Whole blood Mn level was measured by graphite furnace atomic absorption spectrophotometry (AAAnalyst 600, Perkin Elmer, Waltham, MA, USA).

Data Analysis:
9. The treatment of outliers is not detailed.
We did not exclude the outliers of either birth weight or maternal blood manganese level. Although there was a woman with high blood Mn level above 50 μg/L, we could not find a reason to exclude them from the analysis.

10. Mention a second degree polynomial rather than a quadratic term

Thank you for the comments. We replaced “quadratic term” with “second degree polynomial function” as follow:

Therefore, we did not transform it to a log scale. Student’s t-test or ANOVA was performed for unadjusted group differences in maternal blood Mn and birth weight. We assessed the association between maternal Mn level and birth weight by simple regression analysis and then by multiple regression analysis using a quadratic term after adding potential confounders (infant sex, gestational age, maternal education, maternal parity, maternal term weight, maternal income, and maternal age) that were determined from previous studies [13,14,17-19].

P8 L3: Therefore, we did not transform it to a log scale. Student’s t-test or ANOVA was performed for unadjusted group differences in maternal blood Mn and birth weight. We assessed the association between maternal Mn level and birth weight by simple regression analysis and then by multiple regression analysis using a second degree polynomial function after adjusting for potential confounders (infant sex, gestational age, maternal education, maternal parity, maternal term weight, maternal income, and maternal age), which were determined from previous studies [6,14,15,18,19].
Discussion

11. The first paragraph of the discussion looks more like an introduction

+ As you commented, we have deleted the first paragraph of discussion as follows:

High Mn demands of the developing fetus during pregnancy lead to increased blood Mn exposure. Fetuses and neonates could be at higher risk for the toxic effects of Mn because they do not have fully developed homeostatic mechanisms for Mn [20]. Little is known about the effects of deficiency or excess of Mn on infant growth or birth outcome in humans [15]. This study focuses on the relationship between deficiency or high level exposure to Mn in utero and infants' birth weight → Deleted

12. The 3 sentences of the second paragraph carry the same message.

+ As you commented, we revised the second paragraph as follows:

The results of our multicentre cohort study indicated that both low and high blood Mn level of pregnant women is associated with low birth weight of infants. We further identified a curvilinear relationship between maternal blood Mn exposure and infant birth weight, as previously reported [14]. This study provides epidemiologic evidence for adverse effects of both deficiency and excess of Mn during pregnancy from a cohort based on the general population.

↓

P11 L2-5: The results of our multicentre cohort study indicated that both low and high blood Mn level of pregnant women is associated with birth weight below 3000 g of infants. We further identified a curvilinear relationship between maternal blood Mn level and infant birth weight, in accordance with previous report [14].

13. 3rd paragraph, line 8: replace « dividing line » by « point of inflection ».

+ As you commented, we modified 3rd paragraph, line 8 as follows:

They reported an inverted U-shaped relationship with a concentration of 31 μg/L as the
dividing line between maternal blood Mn and birth weight.

↓

**P11 L11:** They reported an inverted U-shaped relationship with a concentration of 31 μg/L as the point of inflection between maternal blood Mn and birth weight.

14. Except for the study in Shanghai (that reports cord serum levels), the comparison studies have very similar levels of exposure (see comment about Introduction), contrary to what is stated.

+We have revised the description as follows:

In Shanghai study [21], cord serum Mn was used as biomarker, with the median maternal and cord serum level of 2.8 μg/dL. The authors examined the relationship between cord serum Mn and birth weight by multiple regression, and did not find a significant association between them. However, because blood Mn level in the cord blood at the time of delivery [14,21] and the biological activity of serum and whole blood Mn is different [15,22], this study cannot be directly comparable to our study.

↓

**P11 L20:** In Shanghai study [21], serum Mn from both cord blood and maternal blood were used as biomarkers, with the median maternal and cord serum level of 2.8 μg/dL and 4.0 μg/dL, respectively. The authors examined the relationship between cord serum Mn and birth weight by multiple regression, and did not find a significant association between them, although they found a negative relationship and nonlinear relationship between cord serum Mn and birth length and Ponderal index, respectively [21].

15. Table 3 Distribution of blood manganese… should be Table 1.

+ As you commented, we revised Table 1 as follows:

**Table 3. Distribution of blood manganese concentration and birth weight by general characteristics of the study subjects**

↓

**Table 1. Distribution of blood manganese concentration and birth weight by general characteristics of the study subjects**
16. *Figure 1: legend for vertical axis should be "odds ratio of LOW birth weight"*

+ According to the suggestion of reviewer 2, we have replaced the Figure 1 to Table 3 as below:

**Table 3. Logistic regression analysis of birth weight below 3000 g and maternal blood Mn concentration**

<table>
<thead>
<tr>
<th>Maternal blood manganese level (µg/dL)</th>
<th>No. of subjects</th>
<th>AOR†*</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12</td>
<td>11</td>
<td>5.82</td>
<td>(0.88-38.47)</td>
<td>0.068</td>
</tr>
<tr>
<td>12 – 19</td>
<td>121</td>
<td>1.50</td>
<td>(0.43-5.17)</td>
<td>0.524</td>
</tr>
<tr>
<td>20 – 27</td>
<td>145</td>
<td>1.42</td>
<td>(0.43-4.65)</td>
<td>0.566</td>
</tr>
<tr>
<td>28 – 35</td>
<td>37</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 36</td>
<td>17</td>
<td>4.34</td>
<td>(0.80-23.49)</td>
<td>0.089</td>
</tr>
</tbody>
</table>

†Adjusted odds ratio, adjusted for infant sex, gestational age, maternal education, maternal parity, maternal term weight, maternal income, and maternal age
Reviewer 2

Major Compulsory Revisions

1. There are many problems with the way the data are interpreted in this manuscript; both data from the literature and their own data. I think it needs to be extensively rewritten. The authors must acknowledge that their observation of a relation between blood Mn and birth weight is very far from proving causation. They need to rewrite the Discussion to reframe their arguments without assuming such causative relation. I believe that caution in interpreting these findings is especially important because the association could be gravely biased.

In accordance with your comment, we have revised the manuscript as follows:

- Title has been changed: “Maternal blood manganese level and birth weight: a MOCEH birth cohort study”
- We revised the manuscript to describe the findings more objectively, e.g., changed ‘exposure’ to ‘maternal blood Mn level’, and ‘effect’ to ‘relationship between maternal blood Mn level and birth weight’ etc.: P2 L6, 13; P4 L10, 18; P8 L10; P11 L4, 6, 8
- We revised the discussion section to differentiate the findings of the study and the intuitions. Conclusions were focused on the objective findings from this study and not extending its relationship to causality: P12 L22; P13 L1

2. A major weakness of this study is the lack of data on iron stores. It is well known that Mn and Fe share uptake mechanisms, and individuals with low iron stores have increase Mn uptake and retention. Early studies reported that workers with anemia had higher Mn levels (e.g. Mena et al. Neurology. 1969; 19:1000). Therefore, women with low iron stores could have higher blood Mn levels, and the lower birth weight could actually be due -- entirely or in part -- to iron deficiency. The potential role of iron should be acknowledged by the authors.

+ We are aware of the interaction between iron and manganese in uptake and in their biologic activities. Unfortunately, in our MOCEH cohort, serum ferritin level was not available, because manganese was not included in the initial hypothesis list of the study. Thus further information on iron intake and anemia and laboratory testing was not available. Study participants were mostly from higher education level and higher socioeconomic status with more awareness on their baby’s health. Prevalence of iron deficiency anemia was very low. We added following sentences in the section presenting the limitation of our study.

This study has a relatively small number of observations, especially at high Mn concentration range, which obscures the relationship at very high levels of maternal blood Mn. In addition, as we did not examine the association between umbilical cord blood Mn levels and birth weight, the temporal relationship between Mn exposure and birth outcome is unclear.
We could not take the point that iron and Mn share uptake mechanisms into account, because the information on the maternal intake of nutritional supplement including iron and calcium, and iron-related health problems including anaemia were not available and prevalence of anaemia was very low. A study conducted in Sweden indicates that Mn level during pregnancy was not related to iron status [Tholin et al., 1995].

3. The authors' argumentation rest on the assumption that high Mn levels during pregnancy = high exposure levels. This is clearly an over simplification, and may even be false. Indeed, population studies have demonstrated that Mn levels in pregnant women are much higher that non-pregnant women, especially towards the end of pregnancy. Non-pregnant women have levels around 8-10 μg/L, whereas levels increase during pregnancy to culminate around 20-40 μg/L at birth. Levels are even higher in cord blood. These high levels are observed in women with no known source of overexposure to Mn. The most logical explanation is that it reflects high physiological needs during gestation. This background should appear in the Introduction of the article. Review the important work from Takser et al. on this topic (Manganese levels during pregnancy and at birth: relation to environmental factors and smoking in a Southwest Quebec population. Environ Res. 2004; 95:119).

+ As you commented, we revised the 1st paragraph has follows:

Pregnant women and infants typically show an increase in blood Mn level, which becomes more prominent in the later phase of pregnancy [8]. Generally, adults maintain stable blood Mn concentrations by Mn homeostasis, which is achieved by regulation of absorption and excretion [3]. Excessive concentrations of Mn are potentially harmful to the foetus due to the underdeveloped ability to eliminate Mn [9,10].

4. Based on these considerations, the title should be changed to remove the term 'exposure'. Clearly, the term 'effect' is also completely inappropriate as well, since it suggests causation. In addition, the references are often inadequate. However, the statistical analyses are appropriate, although I have a few suggestions (see below)
INTRODUCTION
5. I would urge the authors to review their manuscript to ensure proper use of references. It is important that they be pertinent and actually addresses the topic of the statement authors are making. In the 1st paragraph of the introduction, I found several problems with references. For instance, the 1st sentence reads manganese (Mn) is one of the essential nutrients for humans and animals, and is intensively needed during gestation and early infancy [1].

The reference 1 is from Dorman et al. 2005. Well, this animal study showed that in utero exposure to inhaled Mn was associated only to limited increase in fetal Mn levels, limited to the liver and not to blood, lung, brain, or skull cap. This experiment does not address the Mn physiological needs during growth at all.

+ As you commented, we modified the 1st paragraph as follows:

Manganese (Mn) is one of the essential nutrients for humans and animals, and is intensively needed during gestation and early infancy [1].

6. Again in the 1st paragraph of the introduction, the sentence “Mn deficiency may result in poor bone formation, birth defects, and increased susceptibility to seizures [4-6].” References 5 is a review about Mn transport in the brain in the context of high exposure levels causing neurotoxic effects. It does not address Mn deficiency at all.

+ As you commented, we revised the 1st paragraph as follows:

Mn deficiency may result in poor bone formation, birth defects, and increased susceptibility to seizures [3-5].

7. 2nd paragraph, 1st sentence: I would refrain from using the term ‘exposure’ in this context. The presence of manganese in the system is not properly referred to as exposure because...
Mn is an essential nutrient. For instance, we don’t talk about iron exposure, or calcium exposure.

+ As you commented, we modified the 2nd paragraph, 1st sentence as follows:

Mn exposure actually begins before birth as a result of maternal exposure.

8. 2nd paragraph, last sentence “Excessive concentrations of Mn are potentially harmful to the fetus due to the underdeveloped ability to eliminate Mn [9,10].”

This statement is not clearly supported by the literature. Actually, the reference No. 1 (Dorman et al. 2005) supports the contrary, since this an animal study showed increase in Mn levels limited to the liver in fetus exposed in utero exposure to airborne particulates of Mn (there was no increase in fetal blood, lung, brain, or skull cap Mn). Very high Mn levels are found in pregnant women and in cord blood of individuals with no known source of overexposure to Mn, so these high levels probably indicate high physiological needs during gestation.

The reference 9 and 10 provided to support this statement are, again, inappropriate.

+ As you commented, we modified the reference 9 and 10 as follows:

Excessive concentrations of Mn are potentially harmful to the foetus due to the underdeveloped ability to eliminate Mn [9,10].

9. 3rd paragraph, 1st sentence, should read “One of the reported maternal and developmental toxic effects in studies(…)”3rd paragraph, 3rd sentence, “However, these studies were conducted in a population with a high level of environmental exposure,”. Firstly, the studies cited in the previous sentence were conducted in 2 separate groups, one in the U.S. and one in Iran, so it is not a single population. Secondly, no unusual sources of exposure are mentioned in paper on the Iran study, so it is unclear why the authors think this population had high levels of exposure.

+ As you commented, we revised the 3rd paragraph as follows:
One of the reported maternal and developmental toxicities in studies of experimental animals with high Mn exposure is reduced foetal body weight [11,12]. Maternal blood Mn concentration was associated with adverse birth outcomes [13] and birth weight was biphasically associated with maternal blood Mn concentration in an inverted U-shaped dose-response relationship [14]. However, these studies were conducted in a population with a high level of environmental exposure, limiting analysis of the potential relationship between maternal blood Mn and foetal development [15]. There are few reports on the birth outcome of Mn exposure during pregnancy in the general population. Also, effect of the very low level of Mn was less explored.

P4 L16: Mn-related maternal and developmental toxicities have been observed in studies of experimental animals, including reduced foetal body weight and high Mn level [12,13]. Few epidemiologic studies reported the relationship between maternal blood Mn level and birth weight of pregnancy outcome in human [14,15]. In a study in Teheran, Iran, intrauterine growth retardation was linearly associated with lower maternal blood Mn level, but with higher cord blood Mn level [14]. In another study, birth weight was biphasically associated with maternal blood Mn concentration in an inverted U-shaped dose-response relationship [15]. This study was conducted in a population living near a lead and zinc mining site in northeastern Oklahoma, U.S.A., with a potential environmental metal exposure. There are few reports on the birth outcome of Mn level during pregnancy in the general population [16]. Also, the effect of the very low level of Mn was less explored. Little is known about the effects of deficiency or excess of Mn on infant growth or birth outcome in humans [16]. Uncertainty still remains about the degree which Mn level will be adequate for pregnant woman.

10. Last sentence of Data Analysis: “Statistical significance was determined using a p-value of < 0.05, but extended to <0.1, when active interpretation is needed.” I am not sure what active interpretation means.

+ We modified the last sentence of Data Analysis is follows:

Statistical significance was determined using a p-value of < 0.05, but extended to <0.1, when active interpretation is needed.

P8 L16: Statistical significance was determined using a p-value of < 0.05.

RESULTS

11. 2nd paragraph. This sentence makes no sense; it needs to be rewritten. Also, the R² from
model 3 is not really different from that of Model 2 (0.1693 and 0.1631, respectively).

+ In this paragraph, we find that our intention to describe the higher $R^2$ in the second degree polynomial model compared to the linear model was not well documented. We have revised as follows:

When we evaluated the quadratic association of Mn on birth outcome after controlling for possible confounders including infant sex, gestational age, maternal education, maternal parity, maternal term weight, maternal income, and maternal age using a multiple linear regression model, a borderline level of significance was found between blood Mn and birth weight with highest level of $R^2$ (p=0.0541) (Table 2)

↓

P10 L8: When we evaluated the quadratic association of Mn on birth outcome using a multiple linear regression model, a borderline level of significance was found between blood Mn and birth weight (p=0.0541) after controlling for possible confounders including infant sex, gestational age, maternal education, maternal parity, maternal term weight, maternal income, and maternal age (Table 2).

12. You should justify choosing the 3000 g cut-off for birth weight?

+ Categorization of the birth weight by 3000 g was based on the 20th percentile of the study subjects (P8 L12), because most of the neonate in our study was above the ordinary criteria of low birth weight, 2500 g. We have deleted the ‘low birth weight’ from the result and replaced by ‘birth weight below 3000 g’ accordingly: P2 L18; P9 L15, 17, 22; P11 L8.

13. Figure 1 shows OR for birth weight below 3000 g per group of blood Mn levels.

+ As you suggested in #15, we have transformed the Figure 1 to Table 3. (See the response to #15.)

14. Table 1 (mislabeled Table 3) presents different groups for blood Mn. You should use the same groups throughout the paper and justify how the grouping of values was done.

+ As you commented, we used the same categorization of blood Mn level across the manuscript and revised Table 1 as follows:
<table>
<thead>
<tr>
<th>Maternal blood Mn (µg/L)</th>
<th>Birth weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Maternal blood Mn (µg/L)</td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>11 (3.3)</td>
</tr>
<tr>
<td>12-19</td>
<td>121 (36.6)</td>
</tr>
</tbody>
</table>
15. The confidence intervals for the lowest and highest blood Mn are extremely wide, so much that it is not very informative. It must be that there were very few observations. It would be more informative to present a table (not a figure) and add the number of ‘cases’ of birth weight below 3000 g (or another value well justified) per blood Mn group.

+ As you suggested, we deleted the Figure 1 and inserted Table 3 instead.

**Table 3.** Logistic regression analysis of birth weight below 3000 g and maternal blood Mn concentration

<table>
<thead>
<tr>
<th>Maternal blood manganese level (μg/dL)</th>
<th>No. of subjects</th>
<th>AOR†*</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12</td>
<td>11</td>
<td>5.82</td>
<td>(0.88-38.47)</td>
<td>0.068</td>
</tr>
<tr>
<td>12 – 19</td>
<td>121</td>
<td>1.50</td>
<td>(0.43-5.17)</td>
<td>0.524</td>
</tr>
<tr>
<td>20 – 27</td>
<td>145</td>
<td>1.42</td>
<td>(0.43-4.65)</td>
<td>0.566</td>
</tr>
<tr>
<td>28 – 35</td>
<td>37</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 36</td>
<td>17</td>
<td>4.34</td>
<td>(0.80-23.49)</td>
<td>0.089</td>
</tr>
</tbody>
</table>

†Adjusted for infant sex, gestational age, maternal education, maternal parity, maternal term weight, maternal income, and maternal age

16. Table 1 is mislabeled Table 3.

+ We corrected the mislabeled table number as follows:

**Table 3.** Distribution of blood manganese concentration and birth weight by general
characteristics of the study subjects

Table 1. Distribution of blood manganese concentration and birth weight by general characteristics of the study subjects

**DISCUSSION**

17. 1st paragraph: sentence “This study focuses on the relationship between deficiency or high level exposure to Mn in utero and infants’ birth weight.” This is a misleading statement. You observed that the lowest and the highest levels of blood Mn during pregnancy were associated with lower birth weight, but this is clearly no proof that Mn caused these changes in birth weight. I don’t think you can conclude anything about Mn deficiency.

+ As you commented, we revised the first paragraph

This study focuses on the relationship between deficiency or high level exposure to Mn in utero and infants’ birth weight.

↓

P11 L5: Deleted

18. 2nd paragraph. “This study provides epidemiologic evidence for adverse effects of both deficiency and excess of Mn during pregnancy from a cohort based on the general population.” This statement is extremely overreaching. Your study provide no evidence of effect at all; you merely show an association, which could be completely biased, for instance by iron levels.

+ As you commented, we modified the second paragraph as follows:

The results of our multicentre cohort study indicated that both low and high blood Mn level of pregnant women is associated with low birth weight of infants. We further identified a curvi-linear relationship between maternal blood Mn exposure and infant birth weight, as previously reported [14]. This study provides epidemiologic evidence for adverse effects of both deficiency and excess of Mn during pregnancy from a cohort based on the general population...

↓

P11 L2: The results of our multicentre cohort study indicated that both low and high blood Mn level of pregnant women is associated with low birth weight of infants. We further
identified a curvi-linear relationship between maternal blood Mn exposure and infant birth weight, as previously reported [14], from a cohort study based on the general population.

19. 3rd paragraph. “The results of the present study confirm that a high level of blood Mn is associated with low birth weight in full-term infants.” Low-birth weight is defined as below 2500g, and you did not show associations of Mn levels with odds for the prevalence of such birth weights. Please consider using another term than ‘low birth weight’ elsewhere in the manuscript when you refer to your results. Again, the term “confirm” is way too strong and definitive in light of this study.

+ As you commented, we revised the 3th paragraph and deleted “confirm” and “low birth weight” as follows:

The results of the present study confirm that a high level of blood Mn is associated with low birth weight in full-term infants. A high maternal Mn level, over 36 µg/L level, was significantly associated with low birth weight although the relationship between high Mn exposure and low birth weight in our study population was rather weak because the range of blood Mn levels was lower than in other study populations [14].

P11 L6: It shows that a high level of blood Mn is associated with lower birth weight in full-term infants. A high maternal Mn level, over 36 µg/L level, was significantly associated with lower birth weight although the relationship between high Mn level and birth weight below 3000 g in our study population was rather weak.

20. 5th paragraph. “Nonetheless, the results of these human studies suggest that abnormal Mn exposure during early life may affect fetal growth and result in low birth weight.” Your study did not examine “abnormal” exposures to Mn. You have no data on exposures. You only have blood Mn levels, which has not been proven to reflect exposures in this particular group, i.e. pregnant women.

+ As you commented, we modified the 5th paragraph, deleted “abnormal” and “exposure” as follows:

Nonetheless, the results of these human studies suggest that abnormal Mn exposure during early life may affect fetal growth and result in low birth weight.

P12 L21: Deleted
CONCLUSION:
21. Again, high Mn levels are not synonymous with high exposure.

+ As you commented, we revised as follows:

Our study found that both extreme level of maternal Mn level was associated with lower birth weight outcome. A nonlinear association was observed between maternal blood Mn and birth weight. These results may help to determine the reference level for Mn intake in pregnancy.

↓

P13 L5: Our study found that both extreme level of maternal Mn level was associated with lower birth weight outcome in a nonlinear fashion. These results may help to determine the reference level for Mn intake in pregnancy.

Minor essential revisions

1. Table 3: The term ‘unknown’ should be changed for ‘missing’.

+ As you commented, we modified the term ‘unknown’ into ‘missing’ in Table 1.

2. There are too many digits for p-values.

+ As you commented, we presented the digits for p-values to three: Tables 1 - 3