Author's response to reviews

Title: Blood lead level is associated with non-alcoholic fatty liver disease in the Yangtze River Delta Region of China in the context of rapid urbanization

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Author's response to reviews:

Editor's remarks: We agree with the reviewers that more detail is required before your report can document the conclusions that you wish to draw. In regard to the units of measurement, we prefer SI units when appropriate, but other units are also acceptable. You may wish to add a note about conversion of units, so that both SI (molar) and ug/L are provided. Please delete the subheadings in the Results section. The Conclusions need to be reworded. The single sentence that summarizes the study should be expanded to a brief paragraph (slightly longer that the Conclusions in the Abstract). The second sentence does not follow as a logical conclusion from the study and should be removed.

Response: Thank you for giving us the opportunity to revise our manuscript. In the previous version, the reviewers were positive about the relevance and novelty of our work and they have made wonderful suggestions, which we have followed diligently to significantly strengthen our manuscript.
Based on reviewer #2, we have reported lead levels in μg/dL in the revised manuscript. We have also added SI units in the revised manuscript. Moreover, we have deleted the subheadings in the results section and rewritten the conclusions section according to your suggestion.

Reviewer #1: Overall the study was well-defined. However I had the following questions.

[Quality of written English]
Not suitable for publication unless extensively edited.

Response: we have sought the assistance of American Journal Experts to polish our manuscript. Thank you!

[Methods]
1. The study area of the six villages were randomly chosen from YT region, which covers a massive area. Although the authors had mentioned the rapid economical growing of YT region, it is not clear the socio-economic condition of these 6 villages.

Response: Three villages were randomly chosen from Fengcheng community, which is located in Southern Shanghai, 40km away from the downtown. Other three villages were randomly chosen from Xiaoyue community, which is located in eastern Shaoxing city (Zhejiang) adjacent to Hangzhou Bay, 45km away from downtown. With rapid urbanization of the Yangtze River Delta region, residents living in these areas experienced a rapid shift from peasant to citizen. We have added these information in the revised manuscript.

2. Also on page 6, the exclusion criteria were given, however the inclusion criteria are not clear: how the 3427 subject was enrolled or chosen from the population in the first place? Can they represent the population in the Delta region?
Response: Thank you for your good question. Our data source was SPECT-China study. “SPECT-China is a cross-sectional survey on the prevalence of metabolic diseases and risk factors in east China. We used a stratified cluster sampling method to select a sample in the general population. The sampling process was stratified according to rural/urban area and economic development status in Shanghai, Jiangxi Province, and Zhejiang Province. Random sampling was completed before data collection. Adults aged 18 years old and older who were Chinese citizens and lived in their current residence for 6 months or longer were selected and invited into our study. Those with severe communication problems, acute illness, and an unwillingness to participate were excluded from the study. The overall response rate was 90.8%.” More detailed information was described in previous studies [The Journal of clinical endocrinology and metabolism 2015;100(12):4514-4523; Clinical nutrition 2017;36:253-259]

3. Some epidemiological study had indicated that NAFLD is male dominant disease (roughly 2:1),
(e.g., Dig Dis. 2010;28(1):155-61, BMJ Open 2017;7:e014215 etc..)
However in this study, the male proportion in the NFALD groups is only 26% (Table 1). Is that due to this sampling scheme or other reasons? Under the situation, the analysis should be done for men and women separately.

Response: We thank you for your wonderful suggestion and have done the sex-specific analysis in our revised manuscript.

The NAFLD group had significantly greater BLL than normal group in both genders (both P<0.001). In addition, the prevalence of NAFLD significantly increased with the increasing BLL quartiles in men (P for trend=0.032) and women (P for trend=0.001). Compared with women in the lowest quartile of BLL, OR of NAFLD in women in the highest quartile was 1.552 (95%CI 1.111-2.168) (P for trend=0.037) after adjusting for demographic and metabolic factors. In men, this association showed marginal significance (OR 2.090, 95%CI 0.975-4.483, P for trend=0.068)
[Results]

4. BMI appears in the footnote of both table 1 and 2, but not listed in both tables. It is considered to be an important factor and should be listed, unless there are convincing reasons for not taken into account.

Response: Sorry for this careless mistake. We have listed BMI in table 1 and table 2, and also make adjustment for BMI in the logistic regression models.

5. The BLL range (in μg/L) for each quartile should be listed (Table 2 and 3), so that readers can assess the severity. Put "Blood lead level, μg /L" on top of Table 3 is misleading. The odd ratio does not increase by μg/L but by quartile.

Response: Thank you for your good suggestion. We have added the BLL range in Table 2 and Table 3 and deleted “μg/L” on the top of Table 3.

6. Table 2 shows many of the variables are potential confounders, therefore model 3 on Table 3 is more reliable than others.

Response: Thanks for your comment.

[Conclusions]

7. The data and analysis may have supported that elevated BLL was associated with increased prevalence of NAFLD, however did not support the positive association between BLL and rapid urbanization.

In contrast, the authors mentioned that BLL in China has gradually dropped in the past decades, which against the trend of urbanization. E.g., factories in suburban village may also a factor.
To support this argument, the author may check if there was any difference in level of urbanization among the 6 studied villages, and examine the BLL by stratification.

Response: According to the Gross Domestic Product per capita in 2013, Shanghai (14,653 dollars, rank 3/31) is higher than Zhejiang (11,076 dollars, rank 5/31). In addition, Shanghai has higher urbanization level than Zhejiang. Interestingly, residents in Shanghai had significantly higher BLLs than those in Zhejiang [5.54 (4.03-7.78) vs 3.55 (2.50-5.30) μg/dL, \(P<0.001\)], and the prevalence of NAFLD was also significantly higher in Shanghai than in Zhejiang (45.7% vs 35.1%, \(P<0.001\)). These data may partly support the positive association between BLL and rapid urbanization. We have added these information in additional files.

However, GDP per capita in Shanghai and Zhejiang were both significantly higher than the national level (6,807 US dollars). You enlightened us that it could be an interesting area of future study. We will collect data in provinces with lower urbanization level (below the national level) in our future cohort investigation. Thank you!

Reviewer #2: This is an interesting and well written article on lead exposure in the Yangtze River Delta Region in China and its possible association with non-alcohol fatty liver disease. I have the following recommendations and questions:

1. Please provide information on blood lead levels and NAFLD levels by geography, either by village or at least comparing Shanghai to Zhejiang. This information can be added to Tables 1 and 2. Also, consider adjustment in regression models by geography. One concern regarding the association between lead and NAFLD is that either geographical differences or economical/development aspects are related to both increased blood lead levels and NAFLD levels, in which case an association would be observed. In addition to geography, can you please adjust the model for other socioeconomic status variables? I think it is important to discard confounding by other economical factors that would result in both increased lead exposure and NAFLD.

Response: Thank you for your suggestion. We have provided information on blood lead levels and NAFLD by geography (Shanghai/ Zhejiang), and adjusted geography in the logistic regression models.
Moreover, you are definitely right that socioeconomic status is so important that we have listed educational levels of the subjects in Table 1 and Table 2, and further adjusted educational levels in the regression models. The association between BLL and NAFLD remains robust after adjusting for educational level. Thank you!

2. Add more details regarding the laboratory method used to assess blood lead. What is the limit of detection? What percentage of samples was below the limit of detection? What quality control/quality assurance methods are in place? In particular what is the inter-assay coefficient of variation? Does the laboratory participate in quality control programs nationally or internationally?

Response: Thanks for your suggestion. Blood lead levels were tested using atomic absorption spectrometry. Standard curves were established with r>0.9950, and quality control materials were tested before samples were measured. Two quality control personnel participated in the process control. Outliers were detected by duplicate runs. The detection limits for blood lead was 0.1μg/L. None of the samples exhibited values below the detection limits of blood lead. The inter-assay coefficient of variation for lead was 10%. Our central laboratory was certified by the College of American Pathologists.

We have added these details in methods section in the revised manuscript. Thank you!

3. For all the laboratory measures, provide details of which laboratory was used for each analyte.

Response: Thank you for your suggestion. The Blood samples for the fasting plasma glucose (FPG) were centrifuged at the site of collection within 1 hour after collection. Other blood samples were shipped in dry ice within 2-4 h of collection to a central laboratory, which was certified by the College of American Pathologists. Glycated hemoglobin (HbA1C) was assessed by high-performance liquid chromatography (MQ-2000PT, China). FPG, total cholesterol, triglycerides, high density lipoprotein, low-density lipoprotein and ALT were detected using standard laboratory methods. The laboratory methods were consistent throughout the study period. All blood samples were tested using an auto-analyzer at the central laboratory (BECKMAN COULTER AU 680 Germany).
We have described it in detail in the revised manuscript.

4. Can you provide more details on the training provided to the doctors to perform the abdominal ultrasound?

Response: OK! Abdominal ultrasonic (US) examination was performed on all participants by two trained US doctors who were blinded to the clinical and laboratory data, using B-mode ultrasound device (MINDRAY M7, China). The diagnose criteria were based on previous reports [World journal of gastroenterology 2007, 13(10):1579-1584; Gastroenterology 2002, 123(3):745-750]. Before the study began, they tested on several volunteers with different BMI categories and reached a consensus.

5. I recommend that you report lead levels in ug/dL instead of ug/L as ug/dL is the most commonly used unit for lead, the safety standards are generally in those units and most readers are familiar with those units. It will be easier for readers to understand the magnitude of your exposure level and to directly compare with other populations. Also, when referring to data from NHANES, please report the actual values.

Response: Thanks for your kind suggestion. We have reported lead levels in μg/dL in the revised manuscript. Additionally, we reported the actual values in the NHANES study in the discussion section.