Author’s response to reviews

Title: Association of Low-level Blood Lead with Serum Uric Acid in U.S. Adolescents: A Crosssectional Study

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Author’s response to reviews:

Dear Dr. Michael Weber and reviewers,

Thank you for your kind letter and for the reviewers’ comments concerning our manuscript (No. ENHE-D-19-00178). Those comments are valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have made extensive modification on the original manuscript according to the referees’ comments, and carefully proof-read the manuscript to minimize typographical, grammatical, and bibliographical errors. We have already carefully revised and supplied the possible reasons according to the suggestions from all the reviewers. Here below are the detailing changes we have made in response to each criticism or giving based on the reviewers’ comments:

Reviewers’ comments:

Reviewer #1: This publication can add useful information to the literature for the research audience interested in the relationship of blood lead to uric acid. I have the following comments to the authors.

1. General Comment Concerning the Manuscript: In the introduction and elsewhere, there are intermittent, recurrent, and mostly minor issues with English expression. The first sentence is an example. The word "their" should be "its" or even "the." The sentence that begins on line 62 has a syntax error in the initial phrase and subsidiary phrase that is an incomplete thought. There are also misspelled words in parts of the manuscript. The authors could use the support of a grammar/spell checker, or a colleague whose English is stronger.
Response: Authors appreciate reviewer’s valuable comments. We have made extensive modification on the original manuscript according to the your comments, and carefully proof-read the manuscript to minimize typographical, grammatical, and bibliographical errors with the assistance from an individual whose native language is English.

Introduction:

2. A poorly supported statement is "Individuals in the general population are increasingly exposed to lead." There are two problems with the statement. First, it is not correct, the opposite is more correct, although there are some geographic exceptions. And, the referenced article does not support the statement, it features US data and states the opposite of the quoted sentence about the US. The problems could be due to language issues, it is a challenge (and an admirable effort) to write a paper in nonprimary language. The authors are asked to be sure that all references fully support associated statements.

Response: Thank you very much for your suggestions and comments. We apologize for the incorrect description that appeared in the original version. In revised version, we have changed to “Today high dose exposure to lead seldom happens in most developed countries, but chronic low-dose exposures are still a major public health concern”.

3. The statement beginning on line 67 "hyperuricemia is increasingly prevalent among children and adolescents in the United States" 15, 16 is not well supported by at least one of its references (15), which is a prevalence study and does not address temporal change. In general, the authors need to be more precise, about aligning their text with the supportable information form references. (Again, this may be due to language and need NOT be interpreted as something more serious, but is a nevertheless a problem in need of fixing.

Response: Thank you very much for your suggestions and comments. We apologize for the incorrect description that appeared in the original version. We have removed the unreasonable references and changed that sentence. An increasing body of epidemiologic evidence shows that elevated SUA or hyperuricemia is associated with a wide variety of adverse health outcomes, including gout, hypertension, obesity, dyslipidemia, diabetes, cardiovascular disease, intellectual disabilities and all-cause mortality15-19. However, the association between BLL in a very low level and SUA among adolescents remains unclear because of limited data. In addition, elevated SUA is increasingly prevalent among children and adolescents in the United States16. Thus, it is important for researchers to study and understand the association between SUA and continuous lead exposure from the everyday environment.

4. Methods: Line 115: I think the authors are trying to say that Covariates were selected based on sensitivity-tested associations of greater than 10% mean uric acid values. However, I do not know for sure what the sentence actually says, it is not clear.
Response: Authors appreciate reviewer’s valuable comments. The authors apologize for the omission in the original version. We selected the appropriate covariates based on previous studies examining risk factors for SUA as well as adjusting for covariates that, when added to this model, changed the matched odds ratio by at least 10%.


5. Results: The quartile ranges for blood lead are population distinctions without much real difference at the individual level. A blood lead of 1.9 ug/dL (the start of quartile 4) is not that different from the background mean population value. This has implications for methods, and may suggest a decile approach (in addition to the quartile approach already used) as an additional useful comparison.

Response: Thank you very much for your suggestions and comments. According to your suggestion, we further explored the dose-response relation between BLL by a decile approach [≤0.5 (reference), 0.5-0.6, 0.6-0.7, 0.7-0.8, 0.8-0.9, 0.9-1.1, 1.1-1.4, 1.4-1.7, 1.7-2.3, and >2.3 μg/dL] and SUA and elevated SUA. The overall pattern lent further support for a dose-response relation between BLL levels and SUA and elevated SUA (P for trend <0.001).
### Table S2. Association of SUA with BLL levels-a dose-response analysis

<table>
<thead>
<tr>
<th>BLL, μg/dL</th>
<th>SUA, mg/dL</th>
<th>β (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude model</td>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 3</td>
</tr>
<tr>
<td>≤0.5</td>
<td>Reference (0)</td>
<td>Reference (0)</td>
<td>Reference (0)</td>
</tr>
<tr>
<td>0.5-0.6</td>
<td>0.10 (-0.02, 0.22)</td>
<td>0.07 (-0.02, 0.17)</td>
<td>0.08 (-0.02, 0.17)</td>
</tr>
<tr>
<td>0.6-0.7</td>
<td>0.22 (0.10, 0.34)</td>
<td>0.14 (0.05, 0.23)</td>
<td>0.12 (0.03, 0.21)</td>
</tr>
<tr>
<td>0.7-0.8</td>
<td>0.22 (0.10, 0.34)</td>
<td>0.12 (0.03, 0.22)</td>
<td>0.10 (0.01, 0.19)</td>
</tr>
<tr>
<td>0.8-0.9</td>
<td>0.33 (0.21, 0.45)</td>
<td>0.16 (0.07, 0.25)</td>
<td>0.15 (0.06, 0.24)</td>
</tr>
<tr>
<td>0.9-1.1</td>
<td>0.40 (0.30, 0.51)</td>
<td>0.23 (0.14, 0.31)</td>
<td>0.19 (0.11, 0.27)</td>
</tr>
<tr>
<td>1.1-1.4</td>
<td>0.40 (0.30, 0.51)</td>
<td>0.18 (0.10, 0.27)</td>
<td>0.15 (0.07, 0.23)</td>
</tr>
<tr>
<td>1.4-1.7</td>
<td>0.41 (0.29, 0.53)</td>
<td>0.21 (0.11, 0.31)</td>
<td>0.16 (0.06, 0.26)</td>
</tr>
<tr>
<td>1.7-2.3</td>
<td>0.58 (0.47, 0.70)</td>
<td>0.29 (0.19, 0.39)</td>
<td>0.25 (0.15, 0.34)</td>
</tr>
<tr>
<td>&gt;2.3</td>
<td>0.61 (0.50, 0.73)</td>
<td>0.32 (0.23, 0.42)</td>
<td>0.27 (0.17, 0.36)</td>
</tr>
</tbody>
</table>

P for trend $<0.001 <0.001 <0.001 <0.001$

Model 1 was adjusted for sex, age, BMI, race, education status and physical activity;

Model 2 was adjusted for all covariables in model 1 plus adjusted for SBP, DBP, blood cadmium, serum cotinine, hemoglobin, fasting blood glucose, total cholesterol, triglycerides, HDL-C, eGFR, blood urea nitrogen and C-reactive protein;

Model 3 was adjusted for all covariables in model 2 plus adjusted for calcium intake, total monounsaturated fatty acids intake, total polyunsaturated fatty acids intake, total saturated fatty acids intake, total fat intake and total protein intake;

Abbreviations: BLL, blood lead levels; SUA, serum uric acid; CI, confidence interval.
Table S3. Association of elevated SUA with BLL levels-a dose-response analysis

<table>
<thead>
<tr>
<th>BLL, μg/dL</th>
<th>SUA, mg/dL, β (95%CI), P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude model</td>
</tr>
<tr>
<td>≤0.5</td>
<td>Reference (1)</td>
</tr>
<tr>
<td>0.5-0.6</td>
<td>1.23 (0.98, 1.55)</td>
</tr>
<tr>
<td>0.6-0.7</td>
<td>1.40 (1.12, 1.74)</td>
</tr>
<tr>
<td>0.7-0.8</td>
<td>1.57 (1.27, 1.95)</td>
</tr>
<tr>
<td>0.8-0.9</td>
<td>1.50 (1.20, 1.86)</td>
</tr>
<tr>
<td>0.9-1.1</td>
<td>1.84 (1.52, 2.23)</td>
</tr>
<tr>
<td>1.1-1.4</td>
<td>1.97 (1.63, 2.39)</td>
</tr>
<tr>
<td>1.4-1.7</td>
<td>1.83 (1.47, 2.27)</td>
</tr>
<tr>
<td>1.7-2.3</td>
<td>2.66 (2.17, 3.27)</td>
</tr>
<tr>
<td>&gt;2.3</td>
<td>2.69 (2.20, 3.29)</td>
</tr>
</tbody>
</table>

P for trend <0.001 <0.001 <0.001 <0.001

Model 1 was adjusted for sex, age, BMI, race, education status and physical activity;

Model 2 was adjusted for all covariables in model 1 plus adjusted for SBP, DBP, blood cadmium, serum cotinine, hemoglobin, fasting blood glucose, total cholesterol, triglycerides, HDL-C, eGFR, blood urea nitrogen and C-reactive protein;

Model 3 was adjusted for all covariables in model 2 plus adjusted for calcium intake, total monounsaturated fatty acids intake, total polyunsaturated fatty acids intake, total saturated fatty acids intake, total fat intake and total protein intake;

Abbreviations: BLL, blood lead levels; SUA, serum uric acid; OR, odds ratio; CI, confidence interval.
Discussion:

6. The sentence "few population-based studies to date have examined the relationship between BLL and SUA" is not justified. A brief search using key words followed by a reference check of the most relevant findings contradicts the statement. There are plenty of studies that address this topic in general, and this sentence should be omitted. However, the authors do appear to be justified about the thought that there are few papers that do a good job addressing adolescents at this low level of mean population exposure to lead.

Response: Thank you very much for your suggestions and comments. The authors apologize for the omission in the original version. In revised version, the sentence "few population-based studies to date have examined the relationship between BLL and SUA" have been changed to “BLL has been found to be associated with SUA and hyperuricemia in multiple large epidemiologic studies in adults”.

7. The association to lower eGFR is expected, and the authors should discuss predecessor literature in more depth. Krishnan's 2012 PLOS ONE paper from the same data set is helpful in this regard, showing that lower eGFR leads to higher serum blood lead, and should probably be cited. (the association may be bidirectional since lead at much higher levels of exposure is a known nephrotoxin.)

Response: Thank you very much for your suggestions and comments. According to your suggestion, we have discuss predecessor literature in more depth and have cited the references (Krishnan Eswar. Reduced Glomerular Function and Prevalence of Gout: NHANES 2009-10. PLoS ONE. 2012;7(11):e50046.).

BLL is an environmental pollutant that exhibits nephrotoxic activity, frequently evidenced by decreased GFR3. A review indicated that lead contributes to nephrotoxicity, even at BLL below 5 μg/dL3. BLL exposure could cause tubulointerstitial nephropathy, responsible for hyperuricemia and toxic effects on blood nucleo proteins that alter the metabolism of purine, thereby causing hyperuricemia32. The association between BLL and eGFR may be bidirectional33. In our study, we found that the effect of BLL on SUA was more pronounced in populations with lower eGFR. A possible reason for theses results is that lower eGFR may lead to an increase in SUA and elevated BLL by excretion disorders34, 35. Nevertheless, further studies are warranted to elucidate the mechanism underlying the role of BLL in kidney function.

Reviewer #2: This manuscript reports on the association between blood lead measured in US adolescents sampled between 1999 and 2006 and uric acid concentrations in their serum.

The study has a cross sectional design.
The results are of interest as the study includes a large dataset and relatively low levels of blood lead. The observations are done at young age in adolescents, in contrast to other studies that often use adults with higher lead exposure.

1. However the vocabulary and the language used in the manuscript are not of sufficient clarity to allow publication in its present form. The manuscript needs substantial revision because of the language.

Response: Thank you very much for your suggestions and comments. We have made extensive modification on the original manuscript according to your comments, and carefully proof-read the manuscript to minimize typographical, grammatical, and bibliographical errors with the assistance from an individual whose native language is English.

2. Although the statistical analysis are in generally well done, the discussion and conclusion is sometimes questionable.

2.1 There may be problems with reverse causality that should be mentioned.

Individuals with low kidney function may have increased serum uric acid and elevated blood lead levels.

Response: Thank you very much for your suggestions and comments. The authors apologize for the omission in the original version. In revised version, BLL is an environmental pollutant that exhibits nephrotoxic activity, frequently evidenced by decreased GFR3. A review indicated that lead contributes to nephrotoxicity, even at BLL below 5 μg/dL3. BLL exposure could cause tubulointerstitial nephropathy, responsible for hyperuricemia and toxic effects on blood nucleoproteins that alter the metabolism of purine, thereby causing hyperuricemia32. The association between BLL and eGFR may be bidirectional33. In our study, we found that the effect of BLL on SUA was more pronounced in populations with lower eGFR. A possible reason for theses results is that lower eGFR may lead to an increase in SUA and elevated BLL by excretion disorders34, 35. Nevertheless, further studies are warranted to elucidate the mechanism underlying the role of BLL in kidney function.

2.2 As "the effect of BLL on SUA was more pronounced in populations with less than high school level education" the authors gave the advice that "improving education could reduce the impact of BLL exposure on SUA " This suggests a direct causal relationship which is not proven in this study. In contrast, even if lead is a causative factor of the increased uric acid concentration in serum, lower education is an indirectly influencing variable.

Response: Thank you very much for your suggestions and comments. The authors apologize for the omission in the original version. In revised version, these findings suggest that lower education is an indirect influence on the association between BLL and SUA.
2.3 Some of the confounders and covariates that are included in some of the models (model 2, 3 and 4) may also be related to the renal dysfunction and as such may overadjust and hence minimize the association. This should be further discussed as well.

Response: Authors appreciate reviewer’s valuable comments. The effect sizes in different models (model 2, 3 and 4) were similar. In addition, we did a collinear diagnosis and the VIF was less than 5.

Detailed comments:

3. eGFR: in abstract: in full (Estimated glomerular filtration rate), if mentioned for the first time

Response: Thank you very much for your comments. According to your suggestion, we add the full name of eGFR in abstract.

4. line 49-50: lead exposure is not increasing in all countries, eg in many European countries decreasing trends are seen.

Response: Thank you very much for your comments. We apologize for the incorrect description that appeared in the original version. In revised version, we have changed to “Today high dose exposure to lead seldom happens in most developed countries, but chronic low-dose exposures are still a major public health concern”.

5. Lines 128-134: it is not clear why the authors calculate sample weights if they are interested in concentration response associations?

Response: Thank you very much for your comments. NHANES is an ongoing repeated cross-sectional study conducted by the Centers for Disease Control and Prevention (CDC). Our study included all four cycles (1999–2006). Sample weights were used for analyses to account for the complex survey design and non-response of NHANES. We calculated the sample weight based on the following references.


6. Statistical analysis: It may be worthwhile to evaluate also time trends.

Response: Thank you very much for your comments. NHANES is an ongoing repeated cross-sectional study conducted by the Centers for Disease Control and Prevention (CDC). In our present study, we could not evaluate time trends because it was a cross-sectional study. Authors appreciate your valuable comments. Future prospective cohort studies are needed to demonstrate the relationship between BLL and SUA in adolescents.
7. Fig 1: the figures should also contain the individual data points

Response: Thank you very much for your comments and suggestion. Figure 1 was used a generalized additive model (GAM) and smooth curve fitting (penalized spline method) to evaluate the adjusted β-Coefficients for SUA and log-RR for elevated SUA.

To improve the quality and readability of the paper, some other modifications were made in the revised manuscript according to the further comments by the referees.

We really hope this will make it more acceptable for publication.

Best regards,

Yours sincerely,

Prof. Lihua Hu

No. ENHE-D-19-00178