Author's response to reviews

Title: Low level exposure to cadmium increases the risk of chronic kidney disease: analysis of the NHANES 1999-2006

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Author's response to reviews: see over
Sirs,

thank you for the opportunity to revise our paper “Low level exposure to cadmium increases the risk of chronic kidney disease: analysis of the NHANES 1999-2006”.
We found very useful and pertinent the reviewers’ suggestions and criticisms to which we have tried to answer as detailed below.
We feel the paper is now considerably improved and should have reached the high standard required to be published in BMC Public Health. We hope so, but we are also ready to handle the paper according to possible further suggestions and requests from the board.
Thank you for your consideration.
Kind regards

Pietro Manuel Ferraro, MD and Giovanni Gambaro, MD, PhD
Reviewer 1

1. Authors did not report any reference supporting the cut-off of albuminuria used in the study

We agree with the reviewer and we used new gender-specific cut-off for our analysis. Albuminuria is defined as urinary albumin-creatinine ratio $\geq 20$ mg/g for males and $\geq 30$ mg/g for females.

2. Can Authors exclude that association of blood cadmium levels and CKD could be merely dependent on the reduced GFR?

Absorbed cadmium forms a complex with metallothionein-1 and is filtered by the kidneys. So it cannot be excluded that a pre-existent reduction in glomerular filtration rate causes a reduced filtration and elimination from the blood of the cadmium-metallothionein complex. In our discussion we point out that blood cadmium cannot be intended as a sensitive marker of exposition; moreover, its relationship with renal function could be biased in different ways (the one pointed out by the referee being one of them), whereas urinary cadmium is a much more reliable marker of chronic exposure and its levels are not biased by pre-existent renal disease.

3. Greater details must to provided on the correction of serum creatinine. Form the reported formulas (page 5), it appears that in the 1999-2000 survey, measured creatinine was underestimated while it was overestimated in 2005-2006 survey. Is this true? Are these formulas derived from a calibration process? Please provide reference if this is the case. A further concern is related to urinary creatinine. Were the same formulas applied also for correcting creatinine measured in the urine specimens?

Correction of serum creatinine for the 1999-2000 and 2005-2006 surveys is indeed recommended by the analytic sheets that come with NHANES data. To apply formulas for the estimation of the glomerular filtration rate, serum creatinine has to be standardized to reference methods of measurement. As pointed out in the analytic sheets available on the NHANES website (http://www.cdc.gov/nchs/data/nhanes/frequency/lab18_doc.pdf, pag.9), “Serum creatinine assays
on 196 stored specimens from NHANES 1999-2000 were used to determine if serum creatinine needed to be adjusted when compared to a method traceable to a “gold” standard reference method. The Cleveland Clinic Foundation (CCF) laboratory analyzed the serum creatinine specimens using a Roche coupled enzymatic assay (creatiminase, creatinase, sarcosine oxidase, kits # 1775677 and 1775766) performed on a Roche P Module instrument. The Roche method calibrators were traceable to an isotope dilution mass spectrometric method for serum creatinine using standard references methods (NIST SRM 967) and confirmed by analysis of CAP LN-24 linearity set based on NIST assigned values. Serum creatinine by the Roche method was then compared to the original NHANES 1999-2000 measurements which used the Jaffe kinetic alkaline picrate method performed on a Roche Hitachi 917 analyzer. There were significant differences in results between these two measurements. The comparison of values revealed the mean (SD) serum creatinine at NHANES, CCF, and their difference were 0.838 (0.310), 0.996 (0.314), and 0.158 (0.056) mg/dL, respectively (paired t-test, p<0.0001). The Deming regression (adjusting for errors in measurement) for the correction is Standard Creatinine (Y) = 1.013*NHANES Creatinine (X) + 0.147 (r = 0.984)”. The same has been done for the 2005-2006 samples, while the other surveys (2001-2002 and 2003-2004) did not need correction.

We have reported in our paper references to the formulas for correction of serum creatinine suggested in the analytic sheets. Correction was not needed for urinary creatinine since standardization is only required to apply formulas to estimate GFR (e.g. MDRD of CKD-EPI)

4. Throughout the text, data should be expressed not only with median but also with range or interquartile range as Authors did in Table 1

The manuscript now shows medians and interquartile ranges for continuous variables

5. I suggest deleting figures 3-6 since they depict the same results reported in the Tables 2 and 3. To make more legible the results, I also suggest to condense tables 2 and 3 in a single Table. The same for tables 4 and 5. Furthermore, please report uniformly in the tables or in the text the range of quartiles for blood and urinary cadmium

We condensed much of the previous informations showed via tables and figures reducing them to 2 tables and 2 figures
Reviewer 2

1. The CKD-EPI equation has the same four variables as the MDRD Study equation but is more accurate and has been recently shown to be a better predictor of risk in population based studies. I suggest substituting the CKD-EPI equation for the MDRD Study equation in these analyses

We accepted this suggestion and used the CKD-EPI equation to estimate GFR in our manuscript

2. Continuous analyses can be more powerful than categorical. Consider examining the association between eGFR and UACR as continuous variables to Cd levels

As suggested by the referee, the linear association has been performed and showed a significant association between both urinary and blood Cd and eGFR and UACR

3. Table 2 and figure 3 appear to show the same information. Consider revising the tables with the logistic regression so that they show the unadjusted OR and then show the affect of adjustment for each variable/set of variables

We condensed much of the previous informations showed via tables and figures reducing them to 2 tables and 2 figures. Now the table for logistic regression shows the univariate and multivariate models and the ORs for each variable included in the analysis

4. Conclusions appear differ across...

We changed the text accordingly.

5. ROC analyses are helpful for assigning cut-points for diagnostic tests. It may be therefore be informative to indicate the values associated with the maximal sensitivity and specificity? Why was CKD not used for the ROC analyses? It would appear to be helpful to have that information as well or else indicate why analyses were not performed
We added a full analysis in the ROC section with curves for both urinary and blood Cd for the prediction of CKD and albuminuria. We reported in the manuscript the cut-off values for specificity and sensitivity above 70%
Reviewer 3

1. How the sample of subjects was derived was not at all clear. After reading the paper several times it became more obvious that only those subject with blood and urinary cadmium levels were considered from the 1999-2006 NHANES sample. This reviewer was perplexed by the small sample size given that the NHANES sample over that time period was about three times larger. The fact that only a subsample of the NHANES was used only appears in the first paragraph of the Discussion. The authors need to indicate why the sample size was much reduced and what effect, if any, this has on their study.

Indeed, only a subsample of subjects included participating to NHANES underwent analysis for urinary cadmium. However, this subsample was randomly derived so this limitation does not affect the generalizability of our findings. Obviously, if the analysis included more subjects, it would have been beneficial in reducing the random error by restricting the confidence interval of the effects of interest. We have reported better in the text the limitation of the sample population.

2. The authors indicated that urinary cadmium is a better indicator of chronic exposure to this heavy metal. However, they report that both the risk of CKD defined by an estimated GFR <60 and albuminuria are increased based on blood cadmium measurement whereas only increased blood cadmium levels showed an association for both CKD and albuminuria but not urinary cadmium (the risk was increased only for albuminuria). The authors need to further explore the relationship between blood and urinary cadmium and to indicate why urinary cadmium levels were not significantly associated with risk of CKD.

We wrote in Discussion that the association between blood Cd (a marker of acute exposure) and CKD could be biased when analyzed in a cross-sectional survey. Urinary Cd, on the other hand, is a more reliable marker of chronic exposure. In our current paper, we analyzed the interaction effect of blood and urinary Cd on kidney disease and found a significant effect on albuminuria.

3. The authors make much of the importance of cigarette smoke as a source of cadmium and suggest that this exposure may be responsible, at least in part, for the epidemic of CKD.
observed in the U.S. The authors need to consider the increase in CKD in light of declining rates of cigarette smoking in the U.S.

Other factors have to be take into consideration when dealing with the increase in CKD in industrialized countries, e.g. diabetes, obesity, dyslipidemia. The reduction on smoke trends could be counterbalanced by the rise of other risk factors for CKD, but the explanation of the epidemics of CKD was beyond the scopes of our paper

4. The joint effect of elevated blood and urinary cadmium was not explored

In the revised manuscript, we analyzed the interaction effect of urinary and blood cadmium in a multivariate-adjusted model, finding an interaction effect on albuminuria

5. The relevance of the ROC curves was not considered in the Discussion

We expanded the analysis on ROC curves and reported it accordingly in the Discussion

1. Perhaps the most important shortcoming of this paper is that much of the information (at least half of the content of the manuscript) was previously published by Navas-Acien. In that paper a much larger sample size was used. The authors need to make a compelling case that the added information on urinary cadmium justifies a separate manuscript

Urinary cadmium is universally considered as the best marker of chronic exposure to cadmium. The majority of papers dealing with the effects of cadmium exposure on health uses urinary cadmium as a reliable proxy of exposure. We feel that not using urinary cadmium is a shortcoming of the manuscript by Navas-Acien, especially since cross-sectional analyses cannot exclude acute effects, e.g. CKD is defined by a single measurement of serum creatinine. Similarly, elevated blood cadmium can derive from an acute effect instead of reflecting a real exposure.