Title: Bone resorption and environmental exposure to cadmium in children

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

Thank you very much for having given us the opportunity to further re-revise our manuscript: “Bone resorption and environmental exposure to cadmium in children” (MS 7304657605778198). We thank also the Referees for their quality reports.

We carefully addressed all the editorial comments and the comments of the referee. A point by point reply to the comments of the referees is given below.

Reviewer: Yasushi Suwazono

Major compulsory revisions

1. Results (Table 2): The participants should be completely divided in two gender groups and regression analysis should be done in each group.

In the revised manuscript, we report gender stratified regression analysis along with the analysis for the entire population, as the urinary cadmium by gender interaction was not significant. We added the interaction term ($p \geq 0.3$) also to the revised legend of table 2. A consistent pattern of cadmium on the studied bone markers was observed both in girls and boys.

2. Results (urinary Ca): The authors indicated the precipitation of urinary Cd. If so, the urinary Ca in the results should be adjusted for the effect of precipitation based on their own measuring condition. Simultaneously, the authors replied that their low urinary Ca concentrations are in line with recent findings from Berglund, et al (Environ Res. 2011, online) in children. However in the study by Berglund, the urinary Ca was adjusted to specific gravity of 1.012. Therefore, the present results are not comparable immediately with those results due to the difference in adjustment method, unit and precipitation of Ca.

We admit that urinary calcium precipitation might have occurred as our urinary samples were not acidified by EDTA. As Ca precipitation is proportional to the urinary Ca concentration, it is unlikely that this has biased our results (as also indicated by Referee 1). Dr. Suwazono is right that the values from the Berglund et al. publication (Environ Res 2011, online) to which we referred in our first revised letter were not directly comparable with ours because of gravimetry vs creatinine standardization. Our urinary calcium concentration taking into account the differences in gravity and urinary creatinine is on average 33% lower in our study compared with the values reported in 238 children of Bangladesh (age group 8-12) by Berglund et al. (Environ Res 2011). Urinary values of Iranian children (Nikibakhsh et al. Iran J Pediatr 2008) that were standardized for creatinine were about 40% higher than in our population. As our manuscript focuses primarily on urinary deoxypyridinoline, which is a specific marker of bone resorption and this marker is not affected by precipitation, the conclusions of our paper are not influenced by this issue. Nevertheless, we have added this issue in the revised discussion in the paragraph on limitations (page 10 last paragraph).

3. Results (urinary Ca): The authors converted the unit from ‘mg/g creatinine’ to ‘µmol/ mmol crt’. By this conversion, previous 95th percentile of urinary Ca (18.8 mg/g creatinine) in boys corresponds to 53.1 µmol/mmol crt. However, the present 95th percentile of urinary Ca was 401 µmol/mmol crt. Therefore, some conversion error or may exist for urinary Ca in the present results.

The reason for the discrepancy is that in the revised version the values were stratified by gender while in the original submitted manuscript the total population was described.
4. **Results (Urinary DPD):** The authors replied that they have checked the ‘units’ of DPD and changed where necessary. However, revisions of measurement values were not referred in their reply. The authors converted the unit of DPD from ‘ng/g creatinine’ to ‘nmol/ mmol crt’. By this conversion, previous 95th percentile of urinary DPD (6071 ng/g creatinine) in boys corresponds to 1.66 nmol/mmol crt. However, the present 95th percentile of urinary DPD was 695 nmol/mmol crt. Therefore, some conversion error may exist for the urinary DPD in the present results.

See answer comment 3.

We hope that with these clarifications our revised manuscript will be accepted for publication.

We are looking forward hearing from you.

Sincerely Yours,

Muhammad Sughis, Joris Penders, Vincent Hauflroid, Benoit Nemery and Tim Nawrot