Author's response to reviews

Title: Relation between dietary cadmium intake and biomarkers of cadmium exposure

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Version: 2 Date: 18 October 2011

Author's response to reviews: see over
Reviewer's report
Title: Relation between dietary cadmium intake and biomarkers of cadmium exposure
Version: 1 Date: 7 August 2011
Reviewer: Antonio Mutti

Reviewer's report: The manuscript "Relation between dietary cadmium intake and biomarkers of cadmium exposure" by Jelin et al. aims at assessing the relationship between dietary intake and urinary excretion of cadmium, taking into account toxicokinetic modelling.

The study is based on a four-day basket survey and questionnaire on diet in a group of post-menopausal women.

Response: We apologize for any lack of clarity in the manuscript. This study is based on pre-menopausal women (20-50 years old) that participated in a duplicate portion study, not a basket study, i.e. the cadmium content was measured in all foods consumed during four consecutive days. Weighted dietary records were completed in parallel.

Despite its internal validity, the study has a limited external validity (generalizability of results): on the one hand, as the Authors acknowledge, (i) the study group is peculiar within the general population, due to specificities in iron and calcium metabolism, both known to interfere with intestinal absorption of cadmium; (ii) urinary cadmium is not considered to be a good biomarker of exposure until a steady state is reached between intake and renal accumulation, a process requiring months in newly exposed subjects (several studies supporting this notion are available from occupational settings); (iii) therefore, the relationships experimentally found in this study can only make sense if a relative stability of dietary habits is assumed not only over the 4-day study, but also over previous months/years.

Response: We appreciate these comments. (i) Although this study was not aimed to be representative of the population, the prevalence of depleted iron stores (Rybo et al.; 1985) as well as the cadmium intake (Järup et al.; 1998) in these women was very similar to that observed in other populations. This indicates that the women were not different with respect to the major aspects under investigation in the present study. We have now expressed this more clearly in the manuscript (page 11).

(ii) These women can be assumed to be close to steady state as they are exposed to cadmium during their entire life almost only through food and there is no reason to believe that the cadmium exposure via food has changed to any major extent during their lifetime (this has been added, page 10).

(iii) We fully agree that this is fundamental for our assessment and conclusions. Several attempts have been undertaken to ensure that the four days were representative of the women’s usual dietary habits: There was a good agreement between the consumption recorded in the duplicate diets and that reported in the questionnaire (reflecting their usual consumption over time) completed before the study. Further, no significant seasonal variations in the duplicate portions were present (Berglund et al.; 1994). In any case, if dietary habits were changed due to the collection of duplicate portions, this would lead to an underestimation of the observed association between cadmium in diet and urine. This is now emphasized in the manuscript, page 6.
My main concern is that the message conveyed by this paper is potentially misleading, unless the above considerations are discussed and weighted when drawing conclusions.

Response: We appreciate your comments. As recommended we have taken the above considerations into account in the Discussion part of the manuscript.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests: I declare that I have no competing interests.

Reviewer's report

Title: Relation between dietary cadmium intake and biomarkers of cadmium exposure
Version: 1 Date: 23 August 2011
Reviewer: Alfred BERNARD
Reviewer's report: The objective of this study was to determine whether the estimated dietary intake of Cd made by the authors correlates well with the internal dose of Cd assessed on the basis of U-Cd or of B-Cd.

Major concerns
My first major concern is about the small sample size especially as the correlation between U-Cd and dietary Cd intake appears to be mainly driven by a few points. This study was based on relatively non-invasive indicators which can be measured on large populations. Most Cd studies currently published involve a minimum of several hundreds subjects, especially when based on the general population.

Response: We understand the concern about the small sample size and that the correlation appears to be mainly driven by a few points. However, this is a mechanistic study with a considerable number of samples collected. A duplicate portion study is extremely time consuming and very expensive because all duplicates of foods and beverages consumed is collected in buckets and then homogenized before analysis of actual cadmium content. Each women in the study collected daily duplicate diets during four consecutive days, summing up to a total of 228 measured duplicate diets.

To assess the impact of some individuals that could influence the observed correlation, we performed sensitivity analyses to test the robustness of the correlations. After taking out one “outlier” (Cd> 35µg/day), which is the only subject with a deviating cadmium intake, the results remained essentially the same (Figure 2a). The correlation between dietary cadmium and urinary cadmium was 0.32 (p=0.01) instead of 0.38 (p = 0.004), and between model-predicted urinary cadmium and urinary cadmium 0.51 (p=<0.001) instead of 0.54. Thus, as this specific individual was not considered to have an unreasonably high cadmium intake and that the observed association was only affected to a minor degree, we decided to keep all women in the analyses. This information is added to the results page 8, first paragraph).
My second major concern is about the possible confounders and the unavoidable
collinearity between measured variables, a problem which is difficult to resolve with
such a small sample size. Among possible confounders, there is for instance the
urinary creatinine used to adjust for variations in diuresis. We know that this mode of
correction is not entirely reliable and that it leads to an underestimation of U-Cd when
U-creatinine values are very high and to an overestimation of U-Cd when U-creatinine
values are very low. U-creatinine can also vary with age, BMI and diet, further
complicating the situation. BMI is also a possible confounder which apparently has
not been considered (the authors have apparently tested the influence of weight but
not of BMI). To consolidate this study, it would be useful to include a correlation
matrix and to ensure that U-Cd is still correlated with dietary Cd intake when subjected
the data in multivariate analysis. Perhaps this has been done but not clearly reported.

Response: We agree with the reviewer that correcting for urinary creatinine is not entirely
reliable. Therefore, we recalculated all the correlations in the manuscript with the exception of
that involving the one-compartment model, by using the 24-h urinary cadmium instead of the
urinary spot samples. This had very little impact on the results (r=0.35; p=0.007) as compared
to 0.38. (This is now added to the Results section, page 8) However, as the one-compartment
model is only developed for creatinine-corrected urinary cadmium we had to use this method
in the paper. Likewise weight had to be used instead of BMI in the one-compartment model.
However, adjustment for BMI, instead of body weight, did not change the observed
correlation between dietary and urinary cadmium. The partial Pearson correlation between
dietary cadmium and urinary cadmium was 0.42 (p=0.002) when controlling for age, BMI
and serum ferritin as compared to 0.43 (p=0.001) when controlling for age, weight and serum
ferritin. Thus, we consider weight to account for similar confounding as BMI in these women.
The adjustment for BMI has now been added to the manuscript, page 8.

Third, the authors base their manuscript on the assumption that U-Cd reflects the Cd
body burden. One way to check this assumption is to show that U-Cd
correlates indeed with age after adjustment for the dietary intake of Cd, the
serum ferritin level and other possible covariates.

Response: We appreciate this suggestion. Indeed, the urinary cadmium correlates with age
after adjustment for the dietary intake of cadmium, the serum ferritin level and BMI.

<table>
<thead>
<tr>
<th></th>
<th>Pearson correlation coefficients</th>
<th>P</th>
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<tr>
<td>U-Cd and age</td>
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<tr>
<td>U-Cd and age,</td>
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<tr>
<td>Partial variable: D-Cd</td>
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<tr>
<td>U-Cd and age</td>
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<tr>
<td>Partial variables: D-Cd, S-fer</td>
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<tr>
<td>U-Cd and age</td>
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<tr>
<td>Partial variables: D-Cd, S-fer, BMI</td>
<td></td>
<td>0.40</td>
</tr>
</tbody>
</table>


Minor point

Data for U-creatinine should be reported. If no adjustment is made for the
residual association of U-Cd values expressed per g of creatinine, then the
authors should check that their findings persist after eliminating samples with extreme values of U-creatinine (<0.3 and >3 g/l) as this is done routinely for monitoring industrial workers.

Response: We appreciate this comment. As, recommended we now report data for urinary creatinine (Table 1). We also checked that the findings persisted after eliminating four samples of <0.3 g/l U-creatinine (none were above 3 g/L creatinine) (page 8). The correlations did not appreciably change after exclusion of these four women. In addition (see point 2 above), similar results were obtained for 24-hour urine as for creatinine-adjusted urinary cadmium, which support that we can use the latter in our calculations (page 8).

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests: None

References:

