Author's response to reviews

Title: Dietary cadmium intake and risk of prostate cancer: A Danish prospective cohort study

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Author's response to reviews: see over
Reviewer: Bettina Julin
Reviewer's report:
This is an overall well written manuscript on the association between cadmium exposure from food and risk of prostate cancer in a Danish cohort. The statistical analyses are appropriate and straight forward. My main concern relates to the lack of adjustments for energy intake.

- Major Compulsory Revisions

1. Associations between components of the diet and disease cannot be considered primary effects if they are simply the results of differences in total energy intake between cases and non-cases (resulting from differences in body size, physical activity and metabolism). Individual differences in total energy intake produce variations in intake of specific dietary components unrelated to dietary composition because the consumption of most nutrients are positively correlated with total energy intake. Thus, energy-adjustment is standard practice in nutritional epidemiology and is based on the concept that the composition of the diet, independent of total caloric intake, is of primary interest. This adjustment also reduces the artificial between person variation introduced by under and over-reporting of food intake by the FFQ. It is recommended that the authors energy-adjust their estimates of cadmium exposure. Alternative they should energy-adjust the dietary cadmium exposure at least in a sensitivity analysis. There are regression models for this e.g. Willett and Stampfer, Am J epid 1986).

Response: We appreciate the concern given by the reviewer and we agree that total energy intake is crucial to take into account in a model, when looking at e.g. fat intake, which is highly correlated with energy intake. However, we also think that it is important to keep in mind that cadmium is not a natural component/nutrient of the diet, but is a toxic contaminant of the food (and other sources), and is not associated or correlated with total energy intake; we therefore think that it is appropriate to look at absolute quantities of cadmium. In general, we consider that an energy-adjusted model is most appropriate for a nutrient-disease association (macronutrients, especially fat), as described in Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol. 1986 Jul;124(1):17-27: “Because intakes of most specific nutrients, particularly macronutrients, are correlated with total energy intake, they may be non-causally associated with disease as a result of confounding by total energy intake”.

However, we appreciate the suggestion given by the reviewer and we therefore tried to energy-adjust the dietary cadmium exposure in a sensitivity analysis, but this did not change results regarding the cadmium and prostate cancer risk association.

Another crucial point is whether dietary exposure to cadmium can be well-assessed using FFQs. The authors don't mention if their estimates of the average daily dietary exposure to cadmium using a FFQ has been compared with another assessment instrument or biological marker (e.g. urinary or blood cadmium concentrations). If yes, what were the results? Is the FFQ in itself validated? Response: We fully agree with the reviewer that it could be interesting to compare our estimates of average daily dietary exposure to cadmium using FFQ with another assessment instrument or biological marker (e.g. urinary or blood cadmium concentrations); however, we did not have this opportunity on our data.
2. Background. The introduction is very long and the paper would gain by making the introduction more focused. **Response:** We appreciate this consideration and we have now cut down the Introduction and redrafted the text in some passages. We feel that we have obtained a more focused Introduction.

3. Background, Second paragraph. It is unclear what PTWI the authors are referring to. EFSA has estimated a TWI and JECFA has estimated a PTM. Please clarify. **Response:** We are sorry about this confusion; we have now reformulated this section. We now refer to the TWI established by EFSA (2.5 µg/kg bw/week).

4. Ascertainment of prostate cancer. I miss a headline for the description of outcome assessment, e.g. "Outcome assessment and follow-up" or "Ascertainment of prostate cancer" or similar. For the paper to read better, this section should be placed after the "Exposure assessment" section, but before the "Statistical analyses" section. **Response:** As suggested by the reviewer, we have now defined a section "Outcome assessment" and placed it in between the sections "Exposure assessment" and "Statistical analyses".

5. The definition of non-aggressive disease needs clarification. **Response:** The definition of prostate cancer aggressiveness is defined in the section Outcome assessment; “the non-aggressive prostate cancer cases are those who did have the relevant information for defining prostate cancer aggressiveness, but who did not meet the criteria of being aggressive prostate cancer”. This information has now been added to the section.

6. Statistical analyses, paragraph 2 and Table 2. The authors state that out of the 1567 total prostate cases, 400 cases were excluded due to missing information on aggressiveness status, which leaves 1167 cases available for subtype classification. In Table 2, however, they report 840 aggressive cases and 427 non-aggressive cases, summing up to 1267 cases. Please explain. **Response:** This was a typo from our side and just to clarify; We have 1567 total prostate cancer cases in this study. Of these 1567 cases, 840 were aggressive and 327 (and not 427 as first stated in Table 2) were non-aggressive and 400 were not classified.

- Minor Essential Revisions.

7. Exposure assessment. The estimation of the daily cadmium exposure from food deserve a bit more detail. For example, were the cadmium concentrations of the specific food items averaged over several samples and approximately how many samples? **Response:** We have now made it more clearer in the “Exposure assessment” section that: The number of samples analysed of each specific food item was decided on the basis of earlier experience concerning the variation in contents of trace elements in that specific food item, such that the number of samples analysed were larger for food items with larger variation in trace element concentration than for food items with lesser variation. Cadmium concentrations of the specific food items were averaged.

In the FFQ, did you ask for the consumption of whole dishes or only individual food items? - when using FoodCalc, did you consider reduction in weight and consequently cadmium concentration for that specific food item? **Response:** In the FFQ we both asked for individual food items and for whole dishes. For whole dishes we broke down the dishes from defined recipes to specific food items – when using FoodCalc we took into account potential reduction of weight (water and fat) during preparation.

When data was not available for certain food items, why did you choose to go forward in time and not backwards? **Response:** We went forward in time (1998-03) as this was the data
that was available to us and as this period is an extension of the actual period under interest (1993-97).

8. Do the authors have data on diabetes status of the participants? Since diabetes is associated with decreased risk of prostate cancer and the dietary advice given to diabetics is likely to lead to an increased exposure to cadmium diabetics could confound the association between cadmium and prostate cancer. **Response:** We have data available on self-reported diabetes at baseline. We have now tested whether diabetes did confound the association between cadmium and prostate cancer; we found that diabetes did not change estimates with respect to dietary cadmium and prostate cancer risk.

9. Ascertainment of prostate cancer. PSA levels have been used to define advanced prostate cancer which is not a common practice everywhere. In addition, the use of PSA 15 as a cut-off does seem a bit low. In a sensitivity analysis, could you use a higher cut point or even exclude PSA from the definition? Furthermore Gleason sum = 7 is included as a criteria for the definition of advanced prostate cancer. Since numerous studies suggest that Gleason 3 + 4 tumors have a better prognosis than Gleason 4 + 3 tumors, it may be interesting to only include the 4 + 3 pattern in the definition of advanced prostate cancer or to exclude Gleason sum = 7 from the definition. **Response:** There is no conventional method of defining prostate cancer aggressiveness and in general many definitions are used. The definition that we use in our study have been carefully selected and evaluated by experienced clinicians in the field and has also been used in previous studies on the DCH cohort (e.g Roswall N, Larsen SB, Friis S, et al. Micronutrient intake and risk of prostate cancer in a cohort of middle-aged, Danish men. Cancer Causes Control. 2013 Jun;24(6):1129-35). As we do not find any significant association in this study for either total prostate cancer, non-aggressive or aggressive, and as we think that our definitions of prostate cancer type are supported, we do not find that testing other definitions is appropriate. In our study, cases with a Gleason score=7 were defined as aggressive regardless of PSA value and TNM stage, as we consider this indicative of the first step in development of aggressive disease. We were not able to distinguish between the patterns 4+3 and 3+4 in our study, therefore we used the total score of 7.

10. Table 3. Is this the results for total prostate cancer? This should be made clearer in the header. **Response:** Correct, this is the results for all prostate cancer cases (total prostate cancer). This has now been made cleared in the header of Table 3.

11. Discussion, first paragraph. The authors state that the study by West et al. (1991) did not find any significant association between dietary Cd exposure and prostate cancer, regardless of age-group or aggressiveness of the disease. In fact, West et al. actually report an association for all tumors among older men (OR 1.8 ;95%CI: 1.1-3.1, comparing the highest quartile of Cd exposure with the lowest). **Response:** West et al (1991) find no significant association between dietary cadmium intake and prostate cancer regardless of prostate cancer type among men aged 45-67 (West et al, Table 8). Same study found no significant association for aggressive tumors among men aged 68-74, but report a significant association for all tumors comparing the highest quartile of cadmium exposure with the lowest for this age group. We have included this information in the Discussion.

12. Typo. Page 9, line 211. Typo. Please rewrite proportional as proportion. **Response:** This typo has now been corrected.
13. Typo. Page 9, line 231. “…and we did not found…” Please revise to “…and we did not find…” 
Response: This typo has now been corrected.

- Discretionary Revisions

14. Exposure assessment, first paragraph. The authors may want to state that they are estimating the average dietary cadmium exposure. 
Response: For clarity, we have now stated in the exposure assessment section that we estimated the average daily dietary cadmium intake.

15. Results, first paragraph. The authors may want to add the number of aggressive and non-aggressive cases here. 
Response: We welcome this suggestion – and the number of aggressive and non-aggressive cases has now been added to this section.

16. A meta-analysis regarding dietary cadmium intake and risk of cancer was recently published. The authors may want to cite this paper: "Cho YA, Kim J, Woo HD, Kang M. Dietary cadmium intake and the risk of cancer: a meta-analysis. PLoS One. 2013 Sep 17;8(9):e75087. doi: 10.1371". 
Response: We appreciate the reviewer’s awareness of this paper, and we have now cited this paper in the discussion section.

Reviewer's report 3
Reviewer: Emily White
This paper reports on a well designed and analyzed cohort study of the association between cadmium intake from diet and prostate cancer risk. It is well written and the results are appropriately discussed.

Minor essential revisions:
1. The authors need to make clearer that the limitation of this study is the exposure assessment. First, they need to report on how well a FFQ can assess cadmium intake from diet. Are there any studies on, for example, cadmium assessed from a FFQ vs toenail cadmium? If not, then cite validity/reliability studies of other minerals from diet, and generalize about how well minerals are measured from a FFQ. Second, one limitation of the FFQ that should be mentioned is that it is not a measure of total cadmium exposure, the likely true exposure of interest, if cadmium exposure also comes from smoking and occupational exposures. 
Response: We are not aware of any study that compares cadmium intake through diet and toenail cadmium levels. Besides that, it should be noted that these two markers of cadmium exposure cannot be expected to be fully correlated as dietary Cd exposure reflect the amount exposed to through the diet whereas biomarkers such as toenail cadmium levels take into account total cadmium exposure. As the Reviewer suggest, we have mentioned in the Discussion that dietary cadmium intake assessed using FFQ is not a measure of total cadmium exposure, which also included cadmium exposure from smoking and occupational exposures. Statistically we were able to adjust for smoking and we performed stratification analyses on smoking status.

2. The authors need to report their results based on other, more strict definitions of aggressive prostate cancer. Most definitions used in epi studies yield a relatively small percent of prostate cancer cases who meet the definition of aggressive, while in this
study, two-thirds of prostate cancer cases were classified as aggressive (840 of 1267 in Table 2). Is this because PSA screening is uncommon in Denmark? Often Gleason 4+3 (or 8+) is considered aggressive, not 7s with pattern 3+4. To my knowledge, few studies have used PSA values to define aggressive nor used the T part of TNM. Some studies have used prostate cancer as cause of death to define aggressive prostate cancer. Perhaps several definitions could be used to be more comparable to other epidemiological studies. Response: Yes, this is correct; we do not have systematic PSA screening in Denmark. Generally, there is no conventional method of defining prostate cancer aggressiveness and in general many definitions are used. The definition that we use in our paper have been carefully selected and evaluated by experienced clinicians in the field and has also been used in previous studies on the DCH cohort (e.g Roswall N, Larsen SB, Friis S, et al. Micronutrient intake and risk of prostate cancer in a cohort of middle-aged, Danish men. Cancer Causes Control. 2013 Jun;24(6):1129-35). As we do not find any association in this study for either total prostate cancer, non-aggressive or aggressive, and as we think that our definitions of prostate cancer type are supported, we do not find that testing other definitions is appropriate.

3. The discussion of reference 27 (Platz et al.) is misleading. Page 5 states “One study indicated that prostate cancer risk was lower in men with high dietary zinc intake, indicating that a high zinc intake may modify a possible harmful effect of cadmium intake (27)” This study is again cited on page 10 as showing a slightly lower prostate cancer risk among men with moderate to high zinc intake, and the paragraph goes on to say the present study and another one (ref 26) did not find effect modification of the cadmium-prostate cancer association by zinc intake. However, the Platz et al. study explicitly examined the toenail zinc-toenail cadmium interaction in prostate cancer risk and found no evidence of interaction (p for interaction =0.9). So: 1) finding a small decrease in risk associated with zinc “intake” does not support a zinc-cadmium interaction, and should not be cited as providing support for that interaction, 2) it should be stated that the Platz et al study looked at the interaction of cadmium with zinc, with no evidence of any interaction, and 3) the Platz study should not be referred to as one of zinc or cadmium “intake” as that implies intake from food. It should be called a study of zinc and cadmium “exposure” or “toenail” zinc and cadmium. Response: We have now re-read the study by Platz et al. We approve that our manuscript may appear to have over-interpreted or partly imprecisely cited the cadmium-zinc study by Platz et al. We have therefore reformulated the passages concerning this paper: we have deleted the passage on page 5 (in order to comply with comment 2 of Reviewer 1, hereby we also comply with your comment). Furthermore, we have rephrased the citation of this reference (Discussion, second paragraph) to: “Another study using toenail cadmium and zinc concentrations found no evidence that the patterns of association between cadmium and prostate cancer differed by concentrations of zinc or vice versa”. We think that the reference is correctly cited in the Discussion, first paragraph, and have therefore kept this unchanged.

Reviewer's report 2
Reviewer: Yu-Sheng Lin
The goal of this study was to investigate the association between dietary cadmium intake and prostate cancer risk in Danish men. Despite the results are not statistically significant, this work have the potential to improve our understanding of cadmium toxicity upon satisfactory revisions.
- Major Compulsory Revisions
1. As exposure misclassification (measurement error) is a major limitation in the current study, it is critical to characterize (or estimate) the magnitudes of variation of dietary cadmium intake (e.g., between- and within-person variation). Such information would help the readers better understand the role of dietary cadmium intake in prostate cancer. **Response:** We fully agree that exposure misclassification is an important potential limitation of this study, which we also discuss in the Discussion section. Ratios of within-person variance could be informative, but we are limited by the facts that we only have a single dietary cadmium intake estimate per person.

2. Besides dietary intake, cigarette smoking is another major source of cadmium exposure. Given that the results from smoking analysis are not as expected, the authors may consider incorporating the number of cigarettes smoked per day, or number of years smoked into the analyses. **Response:** We have now also carried out analyses including the two additional smoking variables: cigarettes smoked per day and number of years smoked in the model, and found that results remained similar as those reported. This has been stated in the paper, Discussion, 4th section.

- Minor Essential Revisions
1. Line 146-147: reference(s) is need for “…Cases with Gleason score #7, PSA >15, T-stage #3, N-stage #1, or M-stage #1 were defined as aggressive…” **Response:** Same definition is used in our previous papers on prostate cancer based on the same cohort, hereunder the study by Roswall N, et al, 2013 (doi: 10.1007/s10552-013-0190-4). We have now added this paper as reference.

2. Please clarify the sentence (line 147-148) that how the dataset used in the analysis was finally selected: “…For cases who did not have complete information available, the records were reexamined by a medical doctor for classification.” **Response:** This sentence was simply included in order to state that for cases where the relevant information for classification of aggressive prostate cancer was not clearly apparent at first review, a thorough review of these records by a medical doctor was necessary to obtain the needed information in the records. We have now reformulated the sentence to “for cases where relevant information for classification of aggressive prostate cancer was not clearly apparent at first review, a thorough review by a medical doctor was conducted to obtain the needed information in the record (Outcome assessment section).”

3. Line 264-268, stratified analyses by BMI was performed to investigate the influence of endogenous hormone exposure. It is not clear whether participants’ BMI changes over time, and if so, whether such changes will affect the findings. **Response:** The BMI values used in this study are calculated from baseline weight and baseline height. This has now been stated in the text and in the header of Table 3. We were not able to examine whether participants’ BMI have changed during the follow up period, since we only had baseline values available for this study.

4. Will the results remain the same using log-transformation of dietary cadmium intake (as a continuous variable)? **Response:** This has now been tested, and we found that log-transformation of the cadmium variable did not change results regarding the cadmium and prostate cancer risk association.
Discretionary Revisions

The authors may consider updating their literature search and be aware of some of the recent relevant publications to support their findings (e.g., Increased risk of cancer mortality associated with cadmium exposures in older Americans with low zinc intake. J Toxicol Environ Health A. 2013;76(1):1-15). **Response: We acknowledge this suggestion, and we have added recent publications to support our findings, hereunder the suggested paper by Lin YS et al and the study by Cho YA et al as suggested by Reviewer 1.**