

Author's response to reviews

Title: A cross-sectional study of the association between persistent organochlorine pollutants and diabetes

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Author's response to reviews: see over

Dear Editors,

Thank you for the opportunity to submit a revised version of the manuscript. Below we give a point-to-point response to the reviewer's comments. Overall we find the reviews to have been constructive and we find that they have helped us to make the revised manuscript a better one as compared to the original one. We hope that it now will be acceptable for publication.

With best regards,

Lars Hagmar, MD, PhD
Professor

Response to comments from reviewers and editors:

“Your revised manuscript should be in strict accordance with our instructions for authors, cf. the pre-acceptance checklist at <http://www.biomedcentral.com/info/edgr-preacceptcheck.asp>. Please upload a copy without track-changes showing. The title page should be formatted according to EH instructions. Please see <http://www.ehjournal.net/info/instructions/>. Please make sure all text stays within the margins, and do not put page breaks between sections. The paper is missing the required list of abbreviations. Please place competing interests and author's contributions before acknowledgements. The tables need to be properly formatted according to EH instructions.”

Re: We have made our best to revise the manuscript according to the instructions. In your instructions to the authors it is said: “If abbreviations are used in the text either they should be defined in the text where first used, or a list of abbreviations can be provided, which should precede the competing interests and authors' contributions.” **As we in the original manuscript had explained all abbreviations in the text, we were a little surprised that you asked also for an abbreviation list. Anyhow, it is now included.**

Kirsten Ohm Kyvik:

General

This is a study of the putative association between type 2 diabetes (T2D) and persistent organochlorine pollutants (POP's).

One cannot help but feeling that the authors are not very experienced in working with diabetes, e.g genes are not mentioned as important aetiological factors, despite the fact that a number of genes are being identified since the completion of the human genome project.

Re: The first sentence of the Introduction of the original manuscript was: “It is widely believed that the increase in incidence of type 2 diabetes mellitus and obesity is the result of a complex interplay between genetic and environmental factors [1].” **Thus, we had not neglected the fact that genetic factors are of importance in the pathogenesis of T2DM. However, the genetics of T2DM are complex and not clearly defined, and several candidate genes have been suggested to contribute to its pathogenesis, which, although found in one population could not be confirmed in other populations, suggesting regional differences in the contribution of T2DM susceptibility genes. Anyhow we have now revised one of the sentences in the Introduction:** “Age, obesity, central adiposity, lack of physical activity, dietary glyceic load, as well as certain genotypic variants are the main factors identified as responsible for the disease.”

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. It is a problem with this study that no attempts of classifying type of diabetes has been done, since it is an attempt to study T2D and POP's. T2D typically takes it beginning in the adult life, but type 1 diabetes (t1D) can be diagnosed at all ages. The authors mentions that two persons are on insulin as single therapy, and consider this as evidence that almost all patients had T2D. These 2 persons constitute 10% of the diabetic population, though. Some sort of classification ought to have been tried, if only based on age, weight and treatment of diagnosis. There are ways to do this as used by epidemiologists. If this is not possible the authors could exclude the two possible t1D patients from analysis.

Re: This is of course a good point. We performed a sensitivity analysis excluding the two diabetics with insulin only therapy, but it showed that the risk estimates were changed with <1 %. This has been included in both Results and Discussion.

2. Why do the authors choose to dichotomize the exposure variables when doing logistic regression? And why do the do analysis for men and women separately. It is quite possible to do bivariate logistic regression with diabetes as the outcome and with both dichotomised (sex) and continuous exposure variables (age and CB153 or p,p'DDE). If this is what the authors do, I apologize and suggest it is explained a little clearer. The rest of the statistical analysis is appropriate.

Re: We appreciate this comment. We now present logistic regression analyses data with respect to continuous exposure data and for the total group before we present gender stratified analyses. There are clear and significant associations for the whole group for both POP markers with respect to diabetes prevalence. We have rewritten Results, the first paragraph of Discussion and the Results section of Abstract.

3. The participants were asked about weight at 25 years of age, and together with current weight the BMI at 15 years was calculated. It has to be kept in mind, though, that the participants have an age where some of them might not have the same height as when they were 25. This could be very shortly commented on.

Re: There must be some misspelling above, but we think that we have got the point. It is correct that we may have slightly underestimated the height at 25 years of age by measuring the current height, but we think that this has only introduced a minor non-differential misclassification. Anyhow, we have now commented on this in the Discussion.

4. In the methods section para 3, there is something wrong with the numbers. An example: 1500 men were asked to participate, 813 of whom did. Of these 510 men were willing to take part in clinical studies. This leaves 687 male non-participants for the questionnaire study and 990 non-participants for the clinical group. How do the authors reach the number 617 male non-participants?

Re: We apologize if have been unclear. The text in the Methods section is now revised and hopefully easier to understand, eg:

“Out of the group of 813 men that responded to the questionnaire there were 617 subjects that did not participate in the clinical examination. The non-participants had similar age distribution (median 62 years, range 49-84) as the 196 participants (median 60, range 49-84).”

Arnold Schechter:

General

The manuscript addresses an important issue and is of sufficient quality to merit publication

Re: Thanks

 Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached) None

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct) The authors use the term "experimental evidence" without defining or elaborating. They should do so.

Re: We appreciate this comments and have specified the statement, also giving the original reference: "Exposure to dioxin has been linked to drastic reductions in glucose uptake in guinea pigs, mice and rats, *in vivo* as well as *in vitro* [4], leading to speculation that chronic low-level exposure to dioxins might be a risk factor for T2DM [5]."

In the introduction, might not the authors wish to add "genetic factors" to their list of "main factors"?

Re: See response to Kyvik.

On page 4, I am not entirely happy with the use of a review article only rather than seminal articles to make a point.

Re: We have now included a number of seminal original articles.

I wish the authors had used more than one PCB congener. Do they have others? If so, could they include these?

Re: No, we have only analyzed for CB-153. The GC-MS technique we use do not allow analysis of e.g. dioxin-like PCBs. We have in the manuscript argued for CB-153 as a very good index biomarker.

I would like to have seen at least some discussion of dioxin like PCBs and none dioxin like PCBs in the manuscript. The authors mention dioxins but do not discuss dioxin toxic equivalents even in their review of the literature.

Re: We appreciate this comment and have revised the manuscript in accordance. In the Introduction the choice of CB-153 as a biomarker has been motivated in more detail:

"We have chosen to use 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) as a biomarker for POP exposure, because it correlates very well ($r \geq 0.98$) with both total PCB concentration in plasma and serum from Swedish subjects [15-16], and with the 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD) equivalent (TEQ) in plasma from PCB ($r=0.89$) as well as the total POP derived TEQ ($r=0.74$) in plasma in American Vietnam veterans [17]."

Moreover in the Discussion we have inserted the following sentence:

"There are no experimental data supporting that di-ortho PCB congeners such as CB-153, will have a diabetogenic effect by themselves, but CB-153 serves as a good proxy marker also for TCDD TEQ and the total POP derived TEQ [17]"

Not having access to medical records is a weakness in their methodology. Why did they not have such access, at least to establish diagnosis but also for BMI at age 25, which seems quite difficult to do from memory if the mean age is in the 60's.

Re: We admit that it is a weakness that we have not confirmed the diagnoses by medical records (see also response to Kyvik above for how to handle this). We don't think that old medical records, if they could be retrieved at all, would provide better information about height and weight at age 25.

I missed the DeVito toxicology studies relating to lipid metabolism and diabetes. I wonder if review of this work might contribute to the manuscript.

Re: Searching in the PubMed for the term “DeVito” combined with either “lipid metabolism” or “diabetes”, did not reveal any publication that we found to be of relevance for the present study.

In stating that diabetes can alter the pharmacokinetics of some drugs it would be helpful to inform the readers as to the specifics.

Re: This now been described in more specific terms: “T2DM can alter the pharmacokinetics of some drugs due to e.g. glycosylation of plasma proteins or displacement by increased plasma levels of free fatty acids, or through deteriorated kidney function [26]”