Reviewer’s report

Title: Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a Cohort Study

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Reviewer: Dania Valvi

Reviewer’s report:

Reviewer comments on manuscript entitled “Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a Cohort Study” by Dr. Panu Rantakokko et al. submitted to the journal of Environmental Health Research.

This is a well written manuscript that evaluates the associations between several environmental contaminants and liver histopathology and function markers in morbidly obese patients that were recruited prior to bariatric surgery and followed up to one year after surgery. Changes in blood concentrations of the environmental contaminants over one year after bariatric surgery are further described. The main strengths of this study include the availability of liver biopsies that are difficult to get in population studies, and of two measurements of numerous contaminants (POPs, PFAAs) and ALT at baseline and at one year of follow-up. The important limitations include the lack of information about changes in weight status prior to recruitment, that are commonly seen in patients prior to bariatric surgery, and also the lack of information of contaminant concentrations in liver tissue that would have permitted to study correlations between liver and blood tissue concentrations and to make more straightforward conclusions about the associations shown. I have some major concerns in relation to the methods and the statistical approach followed that I list more below. I consider that a more clear presentation of the overall causal diagram of the known/hypothesized associations, the repetition and some extension in statistical analysis and a more thorough interpretation of results are required before acceptance for publication.

Major comments:

1. The rational for confounder selection in the multivariate-adjusted models is not clearly explained (page 9). This is a morbidly obese population, however all statistical models were adjusted further for BMI. If BMI is a mediator rather than a confounder in the association between chemical exposures and liver disease, then adjustment for BMI in the models would not be justified. If obesity occurrence was posterior to the alterations in liver function, then adjusting for BMI could lead to collider stratification bias. Also, why adjustment for insulin level was performed in the models? It may be helpful for the authors and also the reader to show a causal diagram of the hypothesized associations between chemical exposures, the outcomes of interest and the additional covariates and
base on this the selection of covariates included in the final models. The authors should also consider adding in the manuscript the effect estimates from models adjusted only for age - this could be presented, perhaps, as supplemental material.

2. Associations between chemical exposure and ALT at baseline and at 12 months after surgery are presented stratified by sex (results section and Table 5) and one of the authors main conclusion is that in women the non-significant negative associations shown at baseline turn to significant and positive at 12 months after surgery. However, a similar pattern for many of the chemicals tested is also observed in men even though associations do not reach the level of statistical significance, which may likely be due to the smaller number of the analysed men compared to women. My suggestion is not to completely disregard the associations shown in men and revise accordingly the text where these findings are described in the sections of results and discussion. Further, it is not clear why associations are presented separately in the two sexes. Did the authors test for statistical interactions according to sex? If associations are not shown to significantly differ by sex, then the authors could consider showing results for the population overall in Table 5 and provide results stratified by sex as a supplementary table.

3. Since information on chemicals and ALT is available both at baseline and 12 months after surgery, why did the authors not evaluate the association between the change in POPs and the change in ALT levels over the period of one year? The comparison of the effect estimates in the models adjusted and unadjusted by the weight change over this period, it would then help to clarify whether the associations observed between chemicals and ALT are explained by the important weight loss noted in participants after the bariatric surgery. Also, did the authors try to adjust the models at 12 months for weight loss during the last year instead of BMI? Weight loss may be a better predictor of the chemical blood concentrations in this case.

4. Study limitations should be addressed in discussion (small numbers, false findings due to multiple testing etc). Importantly, the authors should consider how the lack of information about obesity management prior to recruitment may have influenced findings. Physical activity and diet recommendations as well as medication are commonly administered to patients prior to bariatric surgery – may this explain any of the associations shown - eg the negative associations shown between some chemicals and lobular inflammation at baseline? Patients with a better compliance to their physician recommendation prior to surgery would more likely have lost weight which could have led to both an increase in POP levels in blood and an improvement in liver lobular inflammation.

Other important comments by section:

Abstract

background, lines 26-30: In the abstract background should be provided
information from prior evidence that justifies the study aims. However, as the text is written the justification of the study aims is not clear. A potential link between POP exposure and the risk of non-alcoholic fatty liver disease has indeed been supported by animal studies but it is not clear whether and why this effect would be “especially seen in obese individuals”. Also, the reason why “levels before and 1 year after bariatric surgery induced weight loss were compared” should be justified here.

methods, lines 32-33: Please, consider rephrasing this sentence and further specify the period that this study was conducted, eg “Liver biopsies were obtained from 161 morbidly obese participants of the Kupio Obesity Surgery Study (KOBS) who underwent bariatric surgery between 20??-20???. The study period of biological sample collection is of great interest as decreasing trends in the levels of exposure for most of the chemicals under study have been noted over time.

methods, line 35: Please, specify in methods that blood samples for the chemical determinations were collected twice and the time of sample collection (during the bariatric surgery and 12 months later?). Also, please consider avoiding the general term of “biochemical variables” and specifying the biomarkers evaluated as study outcomes eg, “biomarkers of insulin resistance (insulin, glucose), metabolism (lipids, adiponectin) and liver function (ALT)”. Also, some of the markers listed here are not later included in analyses, why?

results, line 38: Which serum concentrations? The concentrations measured in the samples collected at the time of the surgery or at one year after surgery? Please, specify.

results, line 39-40: It is not clear why the authors describe results only for women in this sentence. Whether these associations were shown to significantly differ in men should be clarified.

Background

Lines 66-69: The use of the POPs and most of the PFAAs evaluated in this study has been banned in developed countries and decreasing time trends of exposure are noted. Perhaps, the authors would like to clarify this.

Lines 69-76: As written it is not clear that the findings described in relation to liver toxicity occur from experimental studies and not from human studies. Please, revise and rephrase accordingly to clarify this.

Lines 80-81, “Serum levels of certain lipophilic POPs have been consistently associated… type 2 diabetes [24], obesity [25] and insulin resistance [26] in cross-sectional epidemiologic studies.”: This is a very general statement that could be expanded and specified. Which POPs have been associated with obesity and diabetes? The direction of the associations (positive or negative?) is also of interest and should be specified. Also, there are several cross-sectional but also prospective studies suggesting that exposure to POPs (mainly DDE and PCBs) is associated with increased risks for obesity and diabetes and perhaps
the authors would like to replace the individual studies cited here by some of the many literature reviews currently available. Prospective studies provide stronger evidence compared to cross-sectional studies as can reassure that exposure occurred prior to the disease, thus the evidence from prospective and not only from cross-sectional studies should be highlighted in the introduction. Further, I would argue that although fairly consistent positive associations between DDE and obesity outcomes have been reported across previous studies, both positive and negative associations have been previously reported for other POPs (e.g. PCBs) and obesity, and associations with diabetes have been much less explored in humans so far. Given the current state of evidence, in my opinion “consistently associated” is a rather strong statement in this case.

Line 84: Why would the elevation in ALT levels linked to PCB exposure be “unexplained”?

Lines 89-90: I understand the rationale for evaluating associations in the most susceptible population subgroups (in this case morbidly obese subjects) however other populations (e.g. the general population) are also “suitable” for evaluating these associations. I think this sentence should be rephrased.

Line 95: The detection of significant correlations between POPs and liver function markers is not enough to support “an association with liver disease”. Were effect estimates calculated in the cited study? Effect estimates adjusted for other factors would be more convincing for the existence of associations between POP exposure and liver function than simple bivariate correlations.

Methods

Lines 105-107: This sentence could be moved at the end of the section entitled “study population”.

Line 110: Please, specify the chronological period of recruitment. The selection criteria of the study and the participation rate of the initially eligible cases should be also stated in methods.

Lines 116-117: Please, specify the percentage of patients with high alcohol consumption that were excluded from analyses. What was the rationale for dropping these cases from analyses should be clarified. If alcohol consumption is considered to be an important confounder for the associations of interest, why did not the authors adjust for this covariate in the models instead of dropping these observations?

Line 120: Please, clarify whether blood samples were collected at fasting or non-fasting conditions? Lipids may be of reduced validity if non-fasting blood has been used for the determinations. Also, more information about samples collected “at baseline” should be provided. Was sample collection performed the exact same day of the surgery? Prior or posterior to the preoperative preparation of patients? If the later is the case, the authors should consider whether medication or liquids administration at the preoperative period may have influenced the biomarker determinations.
Lines 130-135: In the abstract is previously stated that liver biopsies were obtained from 161 subjects, but here it is stated that biopsies were obtained from 119 subjects. The numbers should be revised and corrected accordingly to match throughout the text. Also, later at the end of this paragraph is stated that 105 subjects were finally classified based on histopathology - what about the rest of subjects with a liver biopsy available?

Line 145: “vast majority if samples” meaning >50% of samples?

Line 166: The authors perhaps would like to explain why they have used Kruskal Wallis and not ANOVA tests for continuous variables – Were all continuous variables not normally distributed?

Lines 170-171: Please, explain how the % of change in mean levels was calculated (by resting the mean of baseline concentrations out of the mean of concentrations measured at 12 months and dividing this by the mean concentrations at baseline? For this, concentrations were used in original scale or after log-transformation? etc)

Results

Line 204: The statement in relation to a decrease in BDE209 concentrations does not seem to hold based on the results shown in Table 2. The mean of concentrations is a bit higher at 1 year compared to baseline and the % of change is estimated to be 148%! Thus, concentrations are shown to have increased over time. The mean change seems unexpected though and should be revised. The authors should revise all numbers shown in this table also for other chemicals and make sure that numbers are correct.

Lines 221-223: A similar pattern of negative to positive associations is also shown for men, although associations are non-significant. Comparisons in terms of statistical significance in the associations shown in men versus women are not straightforward due to the important difference in the number of observations in the two subgroups. To evaluate whether significant differences in the associations may exist between the two sexes at baseline or 1 year, effect modification by sex in the associations of interest should be tested in the models.

Other metabolic biomarkers have been listed previously in methods (eg lipids, adiponectin) but are not presented in the results section. It is not clear whether other biochemical outcomes except from ALT were analysed in this study?

Discussion

Lines 237-239: Please, consider using the terms “is associated”/“is less clearly associated” instead of “a strong”/“less strong”, as the meaning of strong is ambiguous.

Lines 240-242: Since effect modification by sex has not been tested, the comparison of effect estimates between women and men is not clear. Please, see also my comments in relation to this more above.
Lines 242-244: It is not clear what would the authors like to say here. How would “weight loss may reveal some of the associations between POPs and liver disease after resolution of NASH”? – Please, rephrase and expand the rational to clarify this.

Lines 279-283: Statistical interactions by sex should be tested to justify a higher susceptibility in women in this study, if indeed there is any.

What are the limitations of this study?

Conclusions

Conclusions should be revised accordingly to the changes made in other sections.

Lines 330-331: The statement “suggesting potential interactions with gender and weight loss” is unjustified here as interactions have not been tested in this study.

Lines 331-332: Positive associations with ALT were shown only at 12 months after surgery and are possibly explained by the important weight loss noted after surgery...

Tables

Table 1: Please, provide for categorical variables also %, not only absolute numbers, to facilitate comparisons.

Table 2: The % of mean change for PFHxA has to be erroneous. Mean and median levels at baseline and 1 year after are identical, thus a 150% of change cannot be justified – please, revise. Also, the % of change in the mean levels for other chemicals seem to be unexpected based on the means shown in Table. For example, the mean concentrations for PFDA are identical (=0,27) but a mean change of 4.4% is then shown. May this be due to the different subgroups analyses at baseline and 1 year later (n=161 versus 118)? The authors should consider showing in this table descriptive statistics only for the subgroup of patients with available concentrations both at baseline and at 1 year – This would permit more straightforward comparisons.

Other minor comments and typographical errors:

Line 66: Please, specify “the consumption of fatty fish [10]”

Line 81: “type 2 diabetes” should be “T2D” as this abbreviation has been already used more above in the text.

Lines 187-188: “p-values <0.05 were considered significant” should be erased as it is repeatedly stated also in the next sentence.

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests