Author's response to reviews

Title: Use of metformin or other antidiabetic drugs is not associated with a decreased risk of thyroid cancer: a case-control study

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Author's response to reviews: see over
Use of metformin or other antidiabetic drugs is not associated with a decreased risk of thyroid cancer: a case-control study

Dear Doctor Solera,

Please find enclosed the revised version of the manuscript “Use of metformin or other antidiabetic drugs is not associated with a decreased risk of thyroid cancer: a case-control study” which we would like to re-submit to the *BMC Cancer*. We carefully addressed the concerns raised by the reviewers. We would like to thank you for giving us the opportunity to re-submit a revised manuscript and the reviewers for their valuable comments to improve our manuscript.

**Reviewer 1**

**Major Compulsory Revisions:**

1. *The results presented in the manuscript do not support their conclusion that “metformin use was not associated with a decreased risk of thyroid cancer” and “none of the other antidiabetic drugs were associated with a change in risk of thyroid cancer”. They did not find any associations. Therefore, the null hypotheses cannot be rejected with a confidence level of 95%, but their sample size is relatively small, i.e., only 70 patients with both diabetes and thyroid cancer. Without a statistical power of >80%, one cannot*
conclude with sufficient confidence that there are indeed no associations. The conclusion on page 12, line 11-12 is not justified.

We agree with the reviewer that we have a rather small sample size on which we based our analyses despite using one of the largest databases worldwide. Therefore, we already stated on page 8, line 13, line 24 and lines 26-27, on page 9, lines 8-9, as well as on page 10, lines 12-13 that the results were not statistical significant. However, if metformin use is indeed not associated with a decreased risk of thyroid cancer, “statistical significance” of a null result could not be reached even with a larger sample; we would only get a tighter confidence interval around the OR of 1.0, providing more reassurance that it’s indeed a null result. In our view, when considering the point estimates of all our various analyses as a whole, there is no suggestion of a decreased risk of thyroid cancer in association with use of metformin despite a certain imprecision of the single risk estimates presented.

However, we reworded the relevant passages in the abstract (page 2, lines 19-21), on page 9, line 10 and page 12, lines 23-25 in order to address the limitation of low statistical power.

2. There are several distinct types of thyroid malignancies: papillary thyroid carcinoma, follicular thyroid carcinoma, anaplastic thyroid carcinoma, medullary thyroid carcinoma and thyroid lymphoma. All these distinct types of thyroid malignancies have unique pathogenic mechanisms and clinical behavior. It is not reasonable to assume they all respond to metformin and other antidiabetic medications in the same way. Although papillary thyroid carcinoma accounts for about 75 to 80% of all thyroid malignancies, the likelihood of being able to detect statistically significant differences will be compromised by the noise created by inclusion of a mixed bag of different malignancies affect the same anatomical site – the thyroid gland.

We are aware of this issue and address this point as one of the limitations of our study in the discussion section (page 11, lines 26-28). Indeed, lack of information on subtype of cancer is an inherent limitation of most observational studies. We would have loved to have a much larger database with more statistical power, with lots of cases of very rare types of thyroid cancer, and with all conceivable details on the histology of these cancers,
but such a database does not exist in the world. We used one of the largest and best databases worldwide for our study and think that the findings contribute to medical knowledge, despite the fact that we can’t address all details. Our results most likely represent risk estimates for differentiated rather than anaplastic thyroid cancer types.

We added a further comment to the discussion page 12, lines 1-2.

3. Page 3, line 27 and page 9, line 25: The PCCL3 cell line is not a thyroid cancer cell line. It is a clonal rat thyroid cell line that requires thyrotropin for growth. It is used in research on thyroid follicular cell function and iodide uptake. The paper by Andrade et al. (reference 24) reported a role of AMPK in the regulation glucose uptake in non-cancerous thyroid follicular cells. This paper is not relevant to thyroid cancer. The speculation that “AMPK activation by metformin could, in theory, lead to increased glucose uptake and thyroid cancer progression” (page 4, line 2) is not justified. The statement on page 9, line 25, “activation of AMPK has also been associated with increased GLUT1 expression in thyroid cancer cells”, is wrong.

We did not state that PCCL3 is a cancer cell line. Andrade related his findings that AMPK activation upregulates GLUT1 and glucose uptake to the observed increased glucose uptake in malignant thyroid cells. We think that Andrade’s paper is interesting and of potential value also in thyroid cancer as such an observation may help to explain that use of metformin was not associated with a decreased risk of (likely differentiated) thyroid cancer. Furthermore, it has been demonstrated that AMPK is upregulated in papillary thyroid cancer. However, more work needs to be done to clarify the role of AMPK activation in thyroid cancer as suggested by these authors (Vidal AP et al. Eur J Endocrinol 2013;169:521-8).

We clarified the association in the discussion section (page 10, lines 3-7).

4. Page 20, line 38 and page 21, line 44: Why the adjusted OR is adjusted only for BMI, smoking and diabetes but not alcohol consumption, hyperthyroidism and goiter, which have significant associations with thyroid cancer risk as shown in Table 1?
As stated in the methods section on page 7, lines 15-17, “we a priori decided to adjust for the potential confounders BMI, smoking, and a recorded diagnosis of diabetes mellitus (or diabetes duration in the sensitivity analysis restricted to diabetic cases and controls) in the multivariate model.” We had to follow the predefined approach as stated in the protocol which we had to submit to the Independent Scientific Advisory Committee (ISAC) for the Medicines and Healthcare products Regulatory Agency (MHRA) database research. Additionally, we assessed the effect of the covariates mentioned by this reviewer on the relative risk estimate by including them one by one in our a priori model. The reason why we did not include these covariates in the a priori defined model is that these variables are not confounders of the association of interest, since they may be related to thyroid cancer but are unlikely related to the likelihood of getting prescriptions for metformin. In addition, since none of these covariates altered the Odds Ratio for thyroid cancer by more than 10%, we did not include them in the final multivariate model.

5. Page 20, Table 2 and page 21, Table 3: There are multiple discrepancies in the data:

Cases with any metformin use: 49 in table 2, but 47 in table 3.

Cases with 1-29 metformin prescriptions: 23 in table 2, but 21 in table 3.

Controls with any metformin use: 208 in table 2, but 254 in table 3.

Controls with 1-29 metformin prescriptions: 116 in table 2, but 129 in table 3.

Controls with =>30 metformin prescriptions: 92 in table 2, but 125 in table 3.

Number of diabetic patients in controls: 365 in table 1, but 419 in table 3.

Controls with any sulfonylurea use: 154 in table 2, but 188 in table 3.

Controls with 1-29 sulfonylurea prescriptions: 77 in table 2, but 97 in table 3.

Controls with =>30 sulfonylurea prescriptions: 77 in table 2, but 91 in table 3.

Controls with any insulin use: 87 in table 2, but 70 in table 3.

Controls with any TZD use: 41 in table 2, but 53 in table 3.

Metformin users in the general analysis of thyroid cancer (table 1 and 2) do not necessarily have to have a diabetes diagnosis; two of them indeed did not. Hence, the
difference of 49 to 47 metformin users in the whole population of thyroid cancer cases and the subgroup of diabetic patients with a diagnosis of thyroid cancer can be explained. The two patients had 5 and 10 prescriptions respectively and thus are counted in the category of 1-29 prescriptions.

The diabetic cases of table 3 were subject to a new matching to (diabetic) controls, as stated on page 7, lines 3-5. Therefore the number of metformin users among the controls does not have to be the same. For the same reason (i.e., the new matching of diabetic controls in the sensitivity analysis, table 3) the number of diabetic patients in controls varies between table 1 and table 3. The same applies for users of other antidiabetic drugs. We checked again all numbers and they are all correct.

6. Page 9, line 12: “Similar to our findings, any use of sulfonylureas……was associated with an increased risk of thyroid cancer…….” Yet the title of the manuscript and the abstract (page 2, line 20) are contradictory and state that none of the other antidiabetic drugs were associated with a change in risk of thyroid cancer.

As this reviewer pointed out in comment 1, we did not reach statistical significant results. Therefore, we correctly concluded that there was no association.

We added a statement to the abstract (page 2, lines 19-21) to say that our results did not reach statistical significance. We also modified the wording on page 9, line 18.

7. Page 9, line 18: The statement, “These results are contradictory to the reported results of his first study [6]”, is wrong. In reference 6, no statistically significant association of metformin use with the risk of thyroid cancer was detected because of low statistical power. Not finding something does not necessarily mean that thing does not exist. Without sufficient statistical power, no conclusions can be drawn. There is no finding to contradict the finding in Tseng’s subsequent redesigned study. The authors are committing the same type of error in logic as described above in Major Concern 1.

We reworded the respective section on page 9, lines 24-26.
Minor Essential Revisions:

*Page 5, line 14: Why is alcoholism excluded and then alcohol intake is analyzed as a factor associated with thyroid cancer risk? How is “alcoholism” defined?*

Alcoholism in our study is defined as pathologic alcohol consumption in contrast to any other but non-pathological, social use. We added this definition on page 5, line 16.

*Page 10, line 6: TZDs do not inhibit DPP-4. The DPP-4 inhibitors are the gliptins.*

The author is right that the DPP-4 inhibitors are the gliptins. However, TZDs also seem to inhibit the dipeptidyl peptidase IV (DPP-4) as found in the reference stated on page 10, line 17.

**Reviewer 2**

1. *The case and control groups were adequate and properly designed. BMI is considered as a risk factors for several types of cancer, including thyroid gland cancer. This study confirmed that finding. Diabetes, although data in this field are scarce, seem to be not significantly associated with thyroid cancer risk. This study presented the same finding. Metformin appeared to has no (or even deleterious) impact on thyroid cancer risk both among all study subjects as well as in diabetic population.*

   However, for the study purpose, in my opinion, only data regarding antidiabetic drugs use among diabetic subjects are relevant and these data are the most important finding of this study (usually people without diabetes do not use such medications). Taking this into consideration, the primary objective of this study should be limited to diabetic population.

   We agree with the reviewer that most patients who use metformin have type 2 diabetes mellitus (DM). It is therefore an option to just run the analysis in cases and controls restricted to diabetes mellitus. However, we also wanted to address the role of diabetes...
mellitus per se as well as the influence of diabetes duration on the risk of thyroid cancer. We therefore decided *a priori* to perform two analyses, and we defined these two analyses in the protocol to ISAC; this in itself forces us to report the findings of both analyses. Running these two analyses allowed us to answer two important questions, i.e. whether we find an association between DM and thyroid cancer, and whether there is evidence that antidiabetic treatment may alter the risk of thyroid cancer. The analysis restricted to diabetic cases and controls strengthens the finding that diabetes mellitus *per se* has no meaningful impact (i.e., bias by indication and time-related bias are likely negligible). We therefore propose not to omit the analysis in the overall population, as we think that these various analyses and approaches add to the information content of this paper.

2. *From this point of view, table 2 is redundant.*

See comment above

3. *According to what was mentioned above, in abstract data for diabetic (and not total) population should be presented*

See comment above

4. *Obesity was associated with increased risk of thyroid cancer in all study population. Additional analysis regarding impact of obesity on this risk among diabetic subjects would be also interesting.*

A BMI of >30 yielded an OR of 1.20 (0.52-11.58) for thyroid cancer risk in the multivariate analysis of the diabetic patients matched to diabetic controls. Thus, it is closely similar to the OR in the whole group of thyroid cancer cases. Since we consider this not to be a major focus of our analysis, we propose to not report this finding in the manuscript, but we are happy to report it here as information to the reviewer.
Reviewer 3

Major Compulsory Revisions

1. Introduction, Page 3, Line 17: Please clarify whether "also in thyroid cancer" refers to a thyroid cancer cell line or to thyroid cancer patients. Based on later mentions of reference number 20, it appears that you are referring to a cell line or cell lines here.

The reviewer is correct in assuming us referring to cancer cell lines for reference 20. We now specified this on page 3, line 18.

2. Materials and Methods, Page 5, Line 11: How do you define "active history" over the three year period? Is there a requirement for number or frequency of visits?

No, there is no defined number of visits. We did not want to only include patients with a certain number of GP visits since omitting healthier patients who may not have needed their GP during the study period would have introduced a bias to our analyses towards sicker patients. The activity we refer to could have been anything from a recording of a diagnosis, a drug prescription, an immunization or a lab value in the CPRD database after January 1988. We added a statement regarding eligibility on page 5, lines 12-14).

3. Materials and Methods, Page 6, Line 8: Were prescriptions typically of the same length? For example, is everyone with 10 prescriptions for a drug taking the drug for the same duration or is it possible that one person had 10 x 3-month prescriptions and another had 10 x 1-month prescriptions? Perhaps it's more standardized in the UK in the US... if so, please ignore!

In the UK the GPs usually issue a prescription for 3 months for patients with a chronic disease. However, this may vary slightly from patient to patient due to individual circumstances. We do have information on pack size, strength and dosing instructions, but this is usually not available for all patients. In the past we analyzed drug exposure in detail and created default values based on an average patient for those patients for whom
detailed information was lacking. In the end we routinely ended up with closely similar risk estimates regardless of what approach we chose. Thus, using the number of prescriptions as proxy for exposure duration has proven to be accurate and valid over and over again, and so we have chosen to take this same approach here again.

4. Tables 2 and 3: In the footnotes for both tables, it is unclear what you mean when you say that the OR was adjusted for "each other." Please clarify.

We thank the reviewer for helping to improve our wording. We reworded the footnotes on page 21, line 27 and page 22, line 33.

Minor Essential Revisions

1. Discussion, Page 9, Line 16: It is unclear to me what "model I" is referring to--something from the Tseng article referenced? Please clarify.

Yes, ‘model I’ is one of the models used by Tseng in his study. However, as we did not want to go into more detail, we indicated ‘model I’ for the interested reader of the Tseng study to find the referenced risk estimate more quickly in the text if a reader is inclined to do so. On the other hand, if we omit ‘model I’, the information would not be specific anymore.


We thank the reviewer for pointing out the missing period.

3. Some inconsistency throughout the article regarding use of antidiabetic vs. anti-diabetic; please make consistent.

We thank the reviewer for pointing out this inconsistency. We now use the term ‘antidiabetic’ throughout the text.
4. Table 1: Word "no" appears to be missing for CHF and IHD.

We thank the reviewer for pointing out the missing words.

Discretionary Revisions

1. Discussion, Page 9, Lines 1 - 3: Since the finding regarding a potential association between metformin exposure and thyroid cancer was insignificant, you might consider softening this statement. For example, "In the relatively small number of patients with long-term metformin exposure, there was some suggestion of a possible association between metformin and thyroid cancer, although this finding was not significant." Not a required changed, it just seems a bit strong for the data presented.

We agree (see also comments of reviewer 1) and changed the wording of the respective passage as suggested by the reviewer (page 9, lines 3-6).

2. Discussion, Page 9, Line 12: Phrase "similar to our findings" regarding exposure to sulfonylureas, similar to the above, seems too strong to me considering that none of the findings were statistically significant.

We agree with the statement of this reviewer and added 'non-significant' on page 9, line 10.

3. The Discussion section seems a bit unfocused and strays from the major point of the study, which was to determine whether use of metformin or other anti-diabetes drugs are associated with thyroid cancer risk. There seems to be a lot of emphasis placed on the other covariates examined and the discussion strays from the diabetes focus. Perhaps a single paragraph describing how your findings regarding other covariates is consistent with the literature would be more appropriate?
We stated at the beginning of the discussion that metformin use was not associated with a statistically significantly altered risk of thyroid cancer. This is in our opinion the main finding of our study and has been initially defined as our primary outcome. We then discuss these results in the light of available observational and mechanistic evidence and move forward to discuss strengths and limitations of the present study. We included information on other variables and their association with thyroid cancer (such as use of statins or NSAIDs) because we think that these results are of interest for the reader, regardless that our primary focus was a different one.

In our opinion, the discussion of potential confounding and study limitations is important in any observational study. Since diabetes mellitus *per se* did not materially alter the risk of thyroid cancer, we did not focus our discussion on this variable but preferred to discuss other variables which may or may not be potential confounders of the association of interest. We hope we were able to show the rationale for building up the discussion the way we did.

4. Your concluding sentence might be a more appropriate tie in to your original objective if you modified it to include both metformin and the other anti-diabetes medications you assessed. For example, "In conclusion, neither metformin nor any other anti-diabetes medication examined was associated with thyroid cancer in this population-based observational study."

We agree and modified the concluding statement as suggested by the reviewer (page 12, lines 21-25).

We hope we were able to address the concerns to your satisfaction, and we would be pleased to see our study published in *BMC Cancer*.

Sincerely,

Christoph R. Meier, PhD, MSc (corresponding author) on behalf of all authors