Author's response to reviews

Title: Serum glucose and risk of cancer: A Meta-analysis

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Author's response to reviews: see over
Response to reviewers:

Reviewer one report:
Here are my comments for the authors
Major Compulsory Revisions:

1. The Authors claimed that 6.1 mmol/L was used as the cut-off for high glucose. Different original studies have different cut-points. Some used tertile, some used quartile and some used quintile. Please make it clearer how the authors achieved to use the cut-point of 6.1 without requesting original data.

RE: The reviewer is right that this cut-off is an issue. We have now clarified the way we categorised serum glucose in greater detail in the Methods section: “The included studies all used different cut off points for glucose levels, some used tertiles, others quartiles or quintiles. For the sake of this analysis all data was dichotomised into ‘high’ and ‘normal’ as close to the 6.1mmol/L cut off as possible by combining groups above and below this level.”

2. As stated in the text, the mechanism by which raised glucose contributes to risk of cancer is not fully established. What are the rationales for considering prostate, colorectal and breast cancer as IGF-I driven cancers, while considering other cancers were not driven by IGF-1?

RE: It is true that this categorisation is still speculative. Therefore, we have now more clearly described our rationale for the selection of ‘IGF-1 driven’ cancers and added several new references to support this in the Methods section: “Although the identification of which cancers are driven by the IGF-1 axis is not entirely elucidated, the cancers for which the most consistent supporting evidence is available are prostate, colorectal and breast cancer [1-4]. Hence, here we considered these as ‘IGF-1 driven’ cancers. Breast, endometrial and prostate cancers were also combined for a separate subgroup of ‘hormone driven’ cancers.”

Please also see our response to comment 6 from reviewer 2.

3. In the study, almost all reported I2 are higher than 75%, including many in the stratified analyses. It indicates that the percentage of total variation across studies or sub-groups due to heterogeneity is very high. The authors should focus on further investigating sources of heterogeneity (such as using meta-regression), rather than reporting the pooled RRs despite the evidence of high heterogeneity.

RE: Heterogeneity is indeed rather high. Hence, we have added some additional analyses to further investigate what potentially drove this.

In the results: “The $I^2$ statistic showed heterogeneity ($I^2 = 92\%; P<0.05$), even though every individual estimate indicated a positive association. Hence, we conducted a ‘remove one’ analysis to gauge each study’s impact; the $I^2$ statistic did not fall below 85%. Next, we conducted a sensitivity analysis using studies which included ‘all cancers’
as the outcome vs those with site specific outcomes. The heterogeneity remained high and the RR did not change drastically. When looking at “All cancers” as an outcome, the RR was 1.21 (95% CI: 1.09-1.34) with an I² of 92%. When combining all site-specific cancers as an outcome, the RR was 1.38 (95% CI: 1.16-1.63) with an I² of 92%. Tumour-specific analyses were performed for the three most commonly studied cancers and resulted in pooled relative risks of 1.09 (95% CI: 0.95-1.25), 1.35 (95% CI: 1.21-1.51) and 1.14 (95% CI: 1.04-1.26), for breast, colorectal and prostate cancer, respectively. The related I² statistic was 74% for breast, 57% for colorectal, and 53% for prostate cancer.

In the discussion:
“The overall results showed a rather large amount of heterogeneity, as suggested by the I² statistic. All of our sensitivity and subgroup analyses showed consistent findings in terms of direction of the association, while the heterogeneity remained high. Only when we conducted tumour specific analysis, the I² statistic reduced. This suggests that heterogeneity is most likely explained by combining studies with different outcomes. However, the consistent finding of a positive association in all our analyses supports the robustness of our findings.”

We also added a contour enhanced funnel plot to further explore potential publication bias.

In the Methods:
“Potential publication bias was assessed with a contour enhanced funnel plot, as well as Beggs Test[5, 6].”

In the Results:
“When assessing publication bias, the funnel plot showed an area of missing studies which includes regions of both low and high statistical significance suggesting that both studies that showed an non-significantly and significantly inverse association between glucose and cancer were missing. Therefore, under the assumption that studies are being suppressed because of a mechanism based on two-sided p-values, publication bias cannot be accepted as the only cause of funnel asymmetry.”

4. It may not be wise to combine all specific cancers together as an overall outcome, especially when serum glucose may have different or opposite effects on different cancers. For example, many previous studies reported that diabetes might reduce prostate cancer risk while diabetes may increase the risk of developing other cancers, such as breast cancer, liver cancer, and colorectal cancer.

RE: Please see our response to comment 3 as well as to comment 5 below.

5. In the discussion, the authors should also compare the findings associated with serum glucose with some major findings associated with diabetes and discuss the reasons for their consistency and inconsistency

RE: Thank you for this suggestion. We have added the following to our Discussion:
“When investigating serum levels of glucose, it is also important to consider diabetes. A bladder cancer-specific study showed that diabetes was associated with
a 30% increased risk (95%CI: 1.18-1.43), which is consistent with the direction of the association found for serum glucose and cancer in our meta-analyses. Other cancer types which also show a positive association with diabetes include pancreatic, endometrial, breast and colorectal cancer [7-10], however an inverse association has been observed for prostate cancer[11]. The latter must be interpreted with caution as diabetics have higher morbidity and mortality from other diseases. There may be competing risks masking their risk of prostate cancer[12]. However, it is important to note that diabetes is a slightly different exposure than serum levels of glucose as diabetic treatments may normalise glucose levels and potentially also affect risk of cancer[13].”

Minor Essential Revisions:
1. It is unclear if all reviewing and coding were done by one person or by two people.

RE: We have added the following clarification into the methods section:
“The literature review and data collection was conducted by DC and reviewed by MVH.”

2. P8. line 3. The I2 statistics for these sex-stratified analyses was 48%, 96% and 19%, respectively. It is unclear which is for which. Please clarify.

RE: This has been clarified as follows: “The $I^2$ statistic for these sex-stratified analyses was 48% for men, 96% for women and 19% where it was not possible to stratify by sex.”

3. P7, Line 12-13. And P8. Line 8-9. Both places mentioned that “Additional cancer site-specific data were also obtained from the MECA N cohort”. It is unclear what additional data were obtained and how the authors used these information.

RE: This has been clarified: “Unpublished data on glucose and risk of breast, prostate, and colorectal cancer was also obtained from the MECAN group, allowing us to use this large dataset in the analysis of all cancers [14].” The duplicate statement on P8 has been removed.

4. P8. Line 9-11. “The pooled relative risk were 1.09 …… with an I2 statistics of 74%, 57%, and 53%, respectively”. Again, it is hard to guess which is for which.

RE: We apologise for the confusion and have now clarified this: “With an $I^2$ statistic of 74% for breast, 57% for colorectal, and 53% for prostate cancer.”
Reviewer two report:

Major Comments:
1. One major problem is the great heterogeneity in the results, which does not seem to go away easily by the stratifications performed. This may be particularly because many studies were limited to one specific cancer, and these were all mixed with "all cancer" studies.

RE: This issue has now been addressed in more detail. Please see our response to comment 3 from Reviewer 1.

2. The authors mention lack of information about diabetes treatment as one weakness of their study. Why don't they do a stratified analysis for studies which did adjust for treatment/diabetes diagnosis?

RE: Thank you for this suggestion. However, when conducting a meta-analysis we used the crude data available and combine these to obtain the pooled risk ratio. If data were available on diabetes by exposure and outcome status in a paper, then we could run a stratified or adjusted analysis. Unfortunately, this information was not available for the majority of studies. We have clarified this limitation more carefully in the discussion: “Future research including adjustment for components such as age, cancer treatment, diabetes (or its treatments), or BMI would be useful in confirming the importance of raised glucose in carcinogenesis.”

3. i) A cut off of 6.1 seems a mixture of different groups with different exposure intensities.

RE: Please see our response to comment 1 from Reviewer 1.

ii) Besides, for non-fasting conditions this is not really "high" blood glucose. Why didn't they also define a different higher cut off (diabetic range) for a secondary analysis? Most studies likely have more categories of exposure.

RE: Thank you for this comment. However, the majority of studies included were based on fasting glucose measurements. Only six did not specify fasting studies which made it impossible to perform a secondary analysis with a higher cut off for non-fasting measurements. We have however clarified this limitation in our Discussion: “Six of the studies included either had mixed or did not specify fasting status. However, exclusion of these studies did not alter the association observed.”

iii) Also I suggest that the authors should have different cut off limits for studies in which fasting status is known.

RE: Please see our response to the previous comment. We have added this to the methods and results sections:

“We also conducted a secondary analysis excluding those studies which did not specify the fasting status of the glucose samples.”
“Including only those with fasting glucose measurements did not have a large effect on the pooled RR (1.32 (95%CI: 1.11-1.57) or the $I^2$ statistic (92%).”

4. The search strategy seems very limited. For example the authors have searched "glucose" while some studies may report this as blood sugar, or malignancy might be used instead of cancer/neoplasm.

**RE:** We apologise if we haven’t explained our search strategy clearly enough. We used MESH (Medical Subject Headings) terms in all of our searches. Hence, the terms blood glucose/ serum glucose also include blood sugar and hyperglycaemia. Similarly, cancer and neoplasm also cover the terms malignant and metastasis, as part of the MESH term. This combined with our rigorous hand searches leave us confident that our search strategy was broad and robust.

5. What was the age range of participants in the studies? Was there any exclusion based on this? Were the studies required to have adjusted for age?

**RE:** Thank you for this question. We did not specify an age range upfront, apart from adult age. We have now added the age distribution of each study in Table 1.

6. I find the classification of cancers into hormone-driven and IGF-1 driven very arbitrary, particularly that they overlap except for one cancer. I think this whole analysis and the conclusions driven from it are too speculative and should be dropped.

**RE:** Please see our response to comment 2 from Reviewer 1. However, if the editor agrees, we are happy to drop these two subgroup analyses.

**Minor Comments:**
1. How many people (and who) assessed the titles for inclusion? How many checked the data?

**RE:** We have added the following clarification into the methods section:
“The literature review and data collection was conducted by DC and reviewed by MVH.”

2. If language was an exclusion criterion, this must be clearly mentioned in methods.

**RE:** We did not limit searches to English language only.

3. The authors mention checking titles, but then jump into abstracts. What happens between these two?

**RE:** This has been clarified as follows: “Initially, titles were reviewed to assess whether they met inclusion criteria. Titles that indicated the study met these criteria progressed to an abstract review. Upon inclusion after this step, the full manuscript was thoroughly checked to evaluate inclusion and exclusion criteria.”

4. The MECAN group has been mentioned in the results, while this must be
explained in the methods, with more detail.

**RE:** We have now moved the following to the Methods section: “*Unpublished data on glucose and risk of breast, prostate, and colorectal cancer was also obtained from the MECAN group, allowing us to use this large dataset in the analysis of all cancers [14].*”
References


