Dear Editor,

Thank you for considering our manuscript: “High-sensitive C-reactive protein and risk of incident type 2 diabetes: a case-control study nested within the Singapore Chinese Health Study”. We appreciate the insightful critiques and comments from you and the reviewers.
In order to facilitate the review process, we provided a point-by-point response to the comments raised by the editorial committee and reviewers (changes are highlighted in yellow in the revised manuscript). Should you have any additional requests or questions, please do not hesitate to contact me. We look forward to hearing from you.

Sincerely yours,

(On behalf of the authors)

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Responses to Reviewers:

Reviewer #1 (Comments to the author)

1. In the article, the following sentence had been repeated twice "A recent meta-analysis of 18 prospective studies found that the overall relative risk (RR) of T2D was 1.26 (95% confidence interval [CI] 1.16-1.37) per 1 log mg/L increment in CRP levels". Kindly check.

Response: Thank you very much for your suggestion. We have changed the sentence to: “The estimate (OR=1.27 [95% CI 1.09-1.48] per 1 log mg/L increment in CRP levels) was consistent with the pooled relative risk reported from a recent meta-analysis [1.26 (95% CI 1.16-1.37); 18 studies]” in the discussion part (Page 10, line 210-212).

2. Shouldn't the control subjects be free of hypertension and dyslipidemia also? How many of them were on antihypertensive and hypolipidemic drugs? The cases and controls had similar LDL cholesterol levels. What could be the reason behind this?
Response: Thank you very much for your suggestion. We acknowledge that hypertension and dyslipidemia could be confounding factors for the association between CRP and risk of diabetes, thus we adjusted for hypertension and lipid profile (TG and HDL) in the model. To control for confounding, we could use three major methods: restriction, matching and multivariate adjustment. Matching and restriction should be done in both cases and controls (e.g., restriction of all participants to only men, or those without hypertension). If only restricting control participants to those without hypertension and dyslipidemia, this would create artificial association given that cases had a large proportion of individuals with hypertension or dyslipidemia. Therefore, we have chosen multivariate adjustment to control for confounding by hypertension and dyslipidemia. Unfortunately, we did not collect information on antihypertensive and hypolipidemic drugs.

As for LDL, many studies have shown that TG and HDL could be more sensitive predictors for incident diabetes rather than LDL. For example, TG and HDL have been included as components of metabolic syndrome. In our study, we also found that TG was higher while HDL was lower at baseline in cases compared to controls. In addition, previous literature has shown that LDL levels are not necessarily elevated in type 2 diabetes (Ochsner J. 2001 3(3):132-137). Therefore, since LDL was not associated with type 2 diabetes in the present study and LDL levels were not directly measured in our study, we decided to remove LDL from the manuscript.

3. The number of subjects in each quartile differs between Table 2 and Table 3. Also, in quartile 1, the number of diabetes subjects reduced from 78 to 66 when stratified by sex. Kindly explain.

Response: Thank you very much for pointing out the difference. In Table 2, we have used the quartiles based on total participants, while in Table 3, we used the sex-specific quartile to better control for the gender differences. We have now clarified this in the table footnote and method section by mentioning that “… and then conducted a stratified analysis by sex using sex-specific quartiles.” (Page 8, line 160).

4. The BMI calculated from the self-reported weight could be biased as the participants may report either their current weight or their earlier one which may be a year before also. So, the BMI calculated could be a bias one and also its association with risk of diabetes. In Table 2 &3, on what basis the authors classified BMI for Model 3.

Response: Thank you very much for your comment. We agree that calculating BMI from self-reported weight is a limitation and therefore we have acknowledged it in the discussion: “In addition, the BMI was calculated from self-reported height and weight” (Page 12, line 258-259). However, the weight was reported at baseline when all participants were free of diabetes.
Therefore, we do not expect the participants to report their weights differently between incident cases (those developed diabetes in the future) and controls. Furthermore, the self-reported weight would not be differential in different CRP groups (the participants did not know their CRP levels at baseline). In other words, the self-reported BMI was more likely to be non-differential misclassification and may actually underestimate the association. The categorical BMI variable in Model 3 was created according to the BMI distribution in the study sample. A previous study has shown a linear association between BMI and T2D risk in the Chinese population in the current study (Diabetes Care. 2009; 32(6): 1104–1106), therefore, we have now changed the categorical BMI to continuous BMI to better account for its confounding effect. We have changed the results and footnotes in Table 2 and Table 3 (Page 23-26).

5. The A1C of the incident T2DM cases seems to be very low (6.8%). What is the range of A1C? Any reason to select cases with low A1C levels?

Response: In this nested case-control study, the A1C levels were measured at baseline when all participants were free of physician-diagnosed diabetes. Therefore, it is expected that the A1C levels were not that high. Among the incident T2D cases, the range of A1C was between 4.8% and 14.6% at baseline.

Reviewer #2 (Comments to the author)

1. The authors have used self-reported BMI which might not accurately reflect the actual BMI. This should be acknowledged as a limitation of the study.

Response: Thank you very much for your suggestion. We have acknowledged it as a limitation: “In addition, the BMI was calculated from self-reported height and weight.” (Page 12, line 260-261).

2. The intra and inter assay coefficients of variation of the blood lipids should be provided in the methods section.

Response: Thank you very much for your suggestion, the intra assay coefficients of variation of TC, TG and HDL are 0.3-0.8%, 0.5-1.0% and 0.6-0.8% respectively, and the inter assay coefficients of variation of TC, TG and HDL are 0.6-1.2%, 0.7-1.3%, and 0.7-1.3% respectively. We have added following sentence in the method part: “the within-assay and between-assay CVs were all less than 1.3%”. (Page 7, line 146)
3. The BMI cut points used in the study were <20.0, 20.0-23.9, 24.0-27.9, ≥28.0 kg/m². The methods section should include details on which international criteria has been adopted to categorize the subjects.

Response: Thank you very much for your suggestion. The categorical BMI variable was created according to the BMI distribution in the study sample. A previous study has shown a linear association between BMI and T2D risk in the Chinese population in the current study (Diabetes Care. 2009; 32(6): 1104–1106), therefore, we have now changed the categorical BMI to continuous BMI to better account for its confounding effect. We have specified in the method section and the footnotes that BMI was adjusted as a continuous variable “…BMI (continuous)…” (Page 8, Line 153). The results were not changed.

4. In Table 1, the subjects are age, gender and dialect matched. Then, there is no necessity to adjust for these factors in table 2. Model 2, 3 and 4 would suffice.

Response: Age was matched on ±3 years, therefore we have adjusted for continuous variable of age to account for its residual confounding effect since it was not perfectly matched. We did not adjust for gender and dialect in model 1 of table 2 as they are perfectly matched.

5. In table 2, model 3, the BMI cut points used were <20.0, 20.0-23.9, 24.0-27.9, ≥28.0 kg/m². However, the average BMI of the cases and controls are 24.8 ± 3.6 and 22.8 ± 3.3 kg/m² respectively. Hence, using the cut point of < 23 and ≥= 23 kg/m² would be more appropriate.

Response: Thank you very much for your suggestion, we agree that categorizing the current population using a cutoff point of 23 is more appropriate, and that is why we chose 23 as the cutoff point for the stratified analysis by BMI (Table 4). However, in multivariate adjustment of a confounding variable, binary variable will run the risk that a substantial part of the confounding remains (BMJ 2006 6;332(7549):1080). Therefore, we have adjusted BMI as a continuous variable to better account for the residual confounding, and updated the results (Page 23-26).

6. In tables 3, 4 and 5 also, only Models 2, 3 and 4 need to be retained and the 2 BMI categories used.

Response: Thank you very much for your comments. As we explained in question 4, we think that keeping model 1 is necessary because age was not perfectly matched. In Table 4 when we stratified by BMI, we broke the match between cases and controls and used unconditional
logistic regression, therefore, we additional adjusted for sex and dialect in model 1. Again, we have adjusted for continuous BMI in the revision.

7. The method of measurement of LDL cholesterol is not provided in the methods section. Was it calculated using Friedewald formula?

Response: Yes, LDL was calculated using Friedewald formula. We decided to remove the LDL from the manuscript and kept TG and HDL for dyslipidemia. Many studies have shown that TG and HDL could be more sensitive predictors for incident diabetes rather than LDL (e.g., TG and HDL have been included as components of metabolic syndrome). In our study, we also found that TG was higher while HDL was lower at baseline in cases compared to controls, while LDL levels were not significantly different between cases and controls.

8. A flow diagram on the phases of the study and participant selection could increase clarity of the study design

Response: Thank you very much for your suggestion, we have added the following flow diagram as Supplemental Figure 1.

Reviewer #3 (Comments to the author)

This manuscript titled "High-sensitive C-reactive protein and risk of incident type 2 diabetes: a case-control study nested within the Singapore Chinese Health Study" prospectively examined the relation between plasma levels of CRP and risk of type 2 diabetes (T2D) among a Chinese population. Type 2 diabetes have a strong inflammatory etiology, but exclusive functional inflammatory biomarker that predicts diabetes is not yet available. CRP is an extensively studied paradoxical biomarker for disease prediction ranging from diabetes to atherosclerosis. Existing literature provides multiple but often contradictory evidence for CRP as a risk factor to develop type 2 diabetes. Despite the many numbers of studies, the associations between type 2 diabetes and CRP levels are inconclusive, and hence it is an important area that is needed to be explored. The strength of the present study is it population cohort from a well-defined demography, and nested case control is an advantage for this study.

Response: Thank you very much for your positive comment on our study. We have revised the manuscript extensively according to your comments listed below.
1. It would be interesting to know the influence of an additional inflammatory marker such as IL-6 on the etiology of T2D, and its correlation with CRP in the study subjects.

Response: Thank you very much for your suggestion. Unfortunately, we did not measure additional inflammatory markers in our blood sample. However, we will look into that in the future.

2. Analysis of previous drug / status infection in cases and controls will be interesting.

Response: Thank you very much for your suggestion. Unfortunately, we did not have information on previous drugs/status infection in cases and controls in baseline questionnaires. There are 38 participants (11 controls and 27 cases) with hs-CRP levels over 10 mg/L, which indicated status of infection. We further conducted a sensitivity analysis excluding these participants, and the associations remain essentially unchanged: in the fully-adjusted model, the OR (95% CI) associated with per 1 log increment in CRP levels was 1.30 (1.11-1.51) in the total sample, 1.11 (0.89-1.37) among incident diabetes (HbA1c <6.5%) and 1.58 (1.26-1.98) among undiagnosed diabetes participants (HbA1c ≥6.5%).

3. Why authors have not included the analysis of blood pressure in this study.

Response: In the baseline questionnaire, we have recorded the information on history of hypertension, but we did not have their blood pressure measurement. Therefore, we have adjusted for the history of hypertension in the analysis.

4. Flow diagram for the methodology will tremendously help the readers.

Response: As responded to reviewer 2, we have added the a flow diagram as Supplemental Figure 1.

5. Why the HbA1C levels in control has been chosen with a cutoff ≤6.0. Does this include the possibility of IGT in control population?

Response: Since undiagnosed diabetes is common in the general population, we selected controls with HbA1c levels ≤6.0 to exclude the possible undiagnosed diabetes cases in the controls. This may include the possibility of IGT in the control, but this is ok in the nested case control studies, as long as the controls were disease free at both baseline and follow-up.
6. Authors can shorten the length of the discussion and may improve redundancy.

Response: Thank you very much for your suggestion. We have revised the discussion accordingly (Page 10-12).

7. Having the description of quartile values in all the table will help readers.

Response: Thank you very much for your suggestion, we have now added the quartile values in Table 3 and 4 (Page 25-26).

8. It will be interesting to know the duration of time between collection of blood for CRP measurement (Follow-up 1) and diagnosis of diabetes.

Response: Thank you very much, as we mentioned in the results, the mean (±SD) duration between blood donation and diagnosis of T2D was 4.0 ± 1.7 years (Page 8, Line 167-168).

9. Can HOMA-IR index can be included in the analysis to assess the role of IR?

Response: Thank you very much for your suggestion. Unfortunately, the glucose and insulin levels in the current study were not fasting samples, therefore, we could not include HOMA-IR index to assess the role of IR.