PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association between fasting blood glucose levels and stroke events: a large-scale community-based cohort study from China
AUTHORS	Zhang, Ya; Gu, Shujun; Wang, Cuicui; Liu, Dong; Zhang, Qiuyi; Yang, Man; Zhou, Zhengyuan; Zuo, Hui

VERSION 1 – REVIEW

REVIEWER	Tian, Lei Stanford University School of Medicine
REVIEW RETURNED	06-May-2021

GENERAL COMMENTS	Zhang et al aimed to investigate the association between FBG levels and stroke risk in a community-based cohort of Chinese adults with and without diabetes, and presented many notable points such as a long follow-up time of 5.5 years, a cohort with 16,113 participants, and 417 incident cases of stroke. The authors performed a prospective analysis using these data to study the association between FPG and stroke. They conducted a meta-analysis of seven studies investigating this association in the general population. They found higher FBG level was associated with increased stroke risk, and imply the potential application of FBG to predict stroke risk in the prospective population. Overall, this study was carefully designed and the results were well organized and presented. I highly recommend for publication with a few minor revisions and clarification. 1. One concern is that there is no HbA1c measurement. Although it has been underlined in the limitation section, some papers reporting the role of HbA1c in distinguishing people with high Cardiovascular risk from those without diabetes should be cited (DOI: 10.1210/jc.2017-00954 and 10.1056/NEJMoa0908359). 2. It would be interesting to report the use of statins in this population. If the data is available, it should be integrated into statistical analysis. If not, it should be stated in the limitation section. 3. I suggest including a flow chart to show the study design to make it easier for readers to understand the overall workflow. 4. There are some typos in the result section of the abstract. It should be "pooled HR: 1.70, 95% CI: 1.27, 2.29; n=7" according to Figure 1.

REVIEWER	Huang, Yuli Shunde Hospital of Southern Medical University, Cardiology
REVIEW RETURNED	16-May-2021

GENERAL COMMENTS	The study by Zhang et al. aimed to examine the FBG levels on subsequent stroke risk in a community-based cohort in China. The results found that higher FBG level was independently associated with an increased risk of stroke in Chinese adults, especially significant in women. The study is strength by its large sample, and long follow-up duration, with statistical power to draw a solid conclusion. I think after revision, this will be excellent paper. I have several major suggestions for revision: 1. The title and the main purpose had clearly stated that they aimed to explore the FBG levels on subsequent stroke risk in a community-based cohort in China. I am so confused that they lump a meta-analysis, including multiple studies from multi-race in the study. This is redundant. Actually, the adding of the meta-analysis, make the paper's main purpose, the method and the discussion are not concise. Furthermore, the part of meta-analysis is not clear written, many important messages are lacking. Therefore, I strongly suggest to delete the part of meta-analysis, just focus on the Chinese cohort. If you insist to include the part of meta-analysis, a comprehensive revision is needed for this part: e.g, the seach stragety, the definition of higher FPG(what do you mean for higher? Per 1 SD? Per 1 mmol/L, or highest quartile vs the lowest quartile? How to reconcile these results in different in the studies?) 2. Abstract: 1) Primary and secondary outcome measures: FBG levels and stroke events. This is incorrect. FBG level is the exposure, not the come in the study. 2) The risk associations were consistent if classifications of FBG levels following the American Diabetes Association and WHO criteria were used. Such statement is unclear for the general readers. 3. Strengths and limitations of this study: Second point: "This study provides the first meta-analysis studying the association between FBG levels and stroke risk in a general population." This is not true. Multiple meta-analyses had evaluated the association. 4. Introduction: 1) "meta-analysis of 58,160 patients with type 2 diabetes in China revealed that intensive blood glucose treatment could not reduce the incidence of stroke compared with conventional treatment." "Although diabetes is closely related to cardiovascular disease (CVD), there is still insufficient data to support the evidence-based medicine proposal of strictly controlling FBG to prevent stroke." These statements did not fit well in the context. Actually, you are not aimed to explore the association between strictly controlling FBG and risk of stroke in T2DM, BUT to explore the association between baseline FPG(even below the cut-point of DM) and the risk stroke. These are totally different two issues. 2) "Therefore, a further study focusing on the relationship between FBG levels and stroke is crucial, which can help us know whether people who are suffering from diabetes, impaired fasting glucose (IFG), insulin resistance, or other people with higher FBG levels should take prevention strategies against stoke". This sentence is unconcise and not clear. Insulin resistance is a mechanism for IFG/DM. 5. Methods: 1) Multiple reference are lacking for the definition of covariates, e.g hypertension, BMI.

	2) What do you mean for “continuous” measurement of systolic blood pressure ≥140mmHg or diastolic blood pressure ≥ 90mmHg? This is not the correct definition of hypertension. 3) Statistical analysis: IFG is defined as an FBG of 6.1-6.9 mmol/L by WHO and an FBG of 5.6-6.9 mmol/L by the American Diabetes Association (ADA). This is not the content of Statistical analysis. Move to the part for definition of exposure. 4) Patient and public involvement: Patients and the public were not involved this research. This is a cohort study. Of course, patients were involved this research. They are just no involved in the design/analysis/writing of the article. 6. Results: 1) present the baseline data according to nomal FPG, IFG and diabetes, at least as a supplementary file. 2). The author tried to emphasize that the risk of stroke was more significant in women than in men. However, P for interaction between the sex are not reported. 7. Discussion: 1) I think it is interesting that even in patients with prediabetes (IFG) defined according to the WHO or ADA criteria, the risk of stroke was both increased. These results support the notion that prediabetes is not only a high risk of progression into diabetes, but also a risk factor for cardiovascular morbidity. Based on this notion, the author should refer these important studies: PMID: 32669282 and PMID: 27881363: these important meta-analyses had reported that prediabetes is a risk factor of CVD (including stroke) and all-cause mortality. PMID: 33769672: this large sample meta-analysis reported prediabetes is associated with the risk of heart failure, another important comorbidity of dysregulation of blood glucose. 2) Limitation: some important risk factors for stroke were not measured and adjusted, e.g, LDL-C, eGFR.

VERSION 1 – AUTHOR RESPONSE

Responses to Reviewers:
Reviewer 1
Zhang et al aimed to investigate the association between FBG levels and stroke risk in a community-based cohort of Chinese adults with and without diabetes, and presented many notable points such as a long follow-up time of 5.5 years, a cohort with 16,113 participants, and 417 incident cases of stroke. The authors performed a prospective analysis using these data to study the association between FPG and stroke. They conducted a meta-analysis of seven studies investigating this association in the general population. They found higher FBG level was associated with increased stroke risk, and imply the potential application of FBG to predict stroke risk in the prospective population. Overall, this study was carefully designed and the results were well organized and presented. I highly recommend for publication with a few minor revisions and clarification.

1. One concern is that there is no HbA1c measurement. Although it has been underlined in the limitation section, some papers reporting the role of HbA1c in distinguishing people with high Cardiovascular risk from those without diabetes should be cited (DOI: 10.1210/jc.2017-00954 and 10.1056/NEJMoa0908359).

Response: Thanks for your suggestion. We have reviewed relevant literatures and added them in the revised manuscript:
"Some reports revealed that HbA_{1c} played an important role in distinguishing people with high Cardiovascular risk from those without diabetes[49 50]." (Please see page 15, lines 350-351)

2. It would be interesting to report the use of statins in this population. If the data is available, it should be integrated into statistical analysis. If not, it should be stated in the limitation section.
Response: We have integrated the use of statins into our statistical analysis, and the findings were essentially unchanged.

3. I suggest including a flow chart to show the study design to make it easier for readers to understand the overall workflow.
Response: Thanks for your suggestion. We have included a flow chart in the supplemental materials (supplemental appendix S1).

4. There are some typos in the result section of the abstract. It should be "pooled HR: 1.70, 95% CI: 1.27, 2.29; n=7" according to Figure 1.
Response: We apologize for these typos. We have carefully corrected them in the revised manuscript. (Please see page 2, line 53)

Responses to Reviewers:
Reviewer 2
The study by Zhang et al. aimed to examine the FBG levels on subsequent stroke risk in a community-based cohort in China. The results found that higher FBG level was independently associated with an increased risk of stroke in Chinese adults, especially significant in women. The study is strength by its large sample, and long follow-up duration, with statistical power to draw a solid conclusion. I think after revision, this will be excellent paper. I have several major suggestions for revision:

1. The title and the main purpose had clearly stated that they aimed to explore the FBG levels on subsequent stroke risk in a community-based cohort in China. I am so confused that they lump a meta-analysis, including multiple studies from multi-race in the study. This is redundant. Actually, the adding of the meta-analysis, make the paper's main purpose, the method and the discussion are not concise. Furthermore, the part of meta-analysis is not clear written, many important messages are lacking. Therefore, I strongly suggest to delete the part of meta-analysis, just focus on the Chinese cohort.
If you insist to include the part of meta-analysis, a comprehensive revision is needed for this part:
e.g, the seach stragety, the definition of higher FPG (what do you mean for higher? Per 1 SD? Per 1 mmol/L, or highest quartile vs the lowest quartile? How to reconcile these results in different in the studies?)
Response: We understand the reviewer's concern, and thank for the suggestion! Although the meta-analysis is not directly related to the community-based cohort in our study, it provides important 'conclusive' information and corroborates our findings. Considering the comments from the editor and other reviewers, we decided to include the result of this meta-analysis in the revised manuscript. However, we have now added some detailed information regarding metaanalysis in the method section, as suggested by the reviewer. (Please see page 7-8, lines148-164)

Abstract:
2. Primary and secondary outcome measures: FBG levels and stroke events. This is incorrect. FBG level is the exposure, not the come in the study.
Response: Thanks for correcting this. We have deleted the 'FBG levels' from the outcome measure section.

“Primary outcome measures: Stroke events.” (Please see page 2, line 42)

3. The risk associations were consistent if classifications of FBG levels following the American Diabetes Association and WHO criteria were used. Such statement is unclear for the general readers.

Response: We apologize for the confusion. We have revised the statement as follows:
“FBG levels of ≥ 7.0 mmol/L was associated with an increased risk of stroke based on two clinical classifications [ADA: 1.68 (1.24, 2.27); WHO: 1.62 (1.21, 2.13)].” (Please see page 2, lines 47-49)

4. Strengths and limitations of this study: Second point: “This study provides the first metaanalysis studying the association between FBG levels and stroke risk in a general population.” This is not true. Multiple meta-analyses had evaluated the association.

Response: Thanks for the correction! We have revised the statement as followed:
“The meta-analysis based on 6 previously published studies and the current study further confirmed the association between fasting blood glucose levels and stroke risk.” (Please see page 4, lines 61-63)

Introduction:

5. “meta-analysis of 58,160 patients with type 2 diabetes in China revealed that intensive blood glucose treatment could not reduce the incidence of stroke compared with conventional treatment.” “Although diabetes is closely related to cardiovascular disease (CVD), there is still insufficient data to support the evidence-based medicine proposal of strictly controlling FBG to prevent stroke.” These statements did not fit well in the context. Actually, you are not aimed to explore the association between strictly controlling FBG and risk of stroke in T2DM, BUT to explore the association between baseline FPG (even below the cut-point of DM) and the risk stroke. These are totally different two issues.

Response: Thanks for your suggestions. We have deleted the inappropriate part and revised the manuscript accordingly as follows:
“Studies have shown that diabetes mellitus can increase the risk of stroke[6 7]. A cohort study involving 510,000 people observed that diabetes significantly increased the risk of ischemic stroke and hematencephalon[7]. A prospective study showed that insulin resistance or diagnosed diabetes can predict the first stroke[8]. However, these studies focus on diabetes status instead of continuous glucose levels as the study exposure, which limits the generalizability of the research findings. Since fasting blood glucose (FBG) is the least interfered by diet[9], the FBG levels are considered as a more reliable tool to measure blood glucose levels than random blood glucose levels[10]. FBG levels have a stronger predictive function for functional prognosis than random blood glucose levels. Therefore, rather than focusing on random blood glucose, it is important to explore the association between long-term average blood glucose and the risk of stroke.” (Please see page 5, lines 76-86)

6. “Therefore, a further study focusing on the relationship between FBG levels and stroke is crucial, which can help us know whether people who are suffering from diabetes, impaired fasting glucose (IFG), insulin resistance, or other people with higher FBG levels should take prevention strategies against stoke”. This sentence is unconcise and not clear. Insulin resistance is a mechanism for IFG/DM.

Response: Thanks for pointing these. We apologize for the confusion. We have removed this statement from the manuscript.

Methods:

7. Multiple reference are lacking for the definition of covariates, e.g hypertension, BMI.

Response: Thanks for pointing these out. We have added relevant references for the definition of hypertension (reference 13) and BMI (reference 16).

8. What do you mean for “continuous” measurement of systolic blood pressure ≥140mmHg or diastolic blood pressure ≥ 90mmHg? This is not the correct definition of hypertension.

Response: Thanks for the comment. We measured the blood pressure three times every 30 seconds, and averaged them as the final measure of blood pressures. We defined hypertension as an average measurement of systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90mmHg according to the WHO definition of hypertension in reference 13. We have revised the statement to make it clearer:

“The criteria for defining hypertension were an average measurement of systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90mmHg[14], diagnosis of hypertension, or antihypertensive medication.” (Please see page 7, lines 137-139)

9. Statistical analysis: IFG is defined as an FBG of 6.1-6.9 mmol/L by WHO and an FBG of 5.66.9 mmol/L by the American Diabetes Association (ADA). This is not the content of Statistical analysis. Move to the part for definition of exposure.

Response: Thanks for your suggestion. We have moved them to the assessments of covariate section.

10. Patient and public involvement: Patients and the public were not involved this research. This is a cohort study. Of course, patients were involved this research. They are just no involved in the design/analysis/writing of the article.

Response: We agree with the reviewer, and we have revised the statement as follows:

“Patients and the public were not involved in the design, conduct, or reporting of this study.” (Please see page 9, lines 194-195)

Results:

11. present the baseline data according to nomal FPG, IFG and diabetes, at least as a supplementary file.

Response: Thanks for your suggestions. We have added the baseline data of FBG, IFG and diabetes in Table 1.

12. The author tried to emphasize that the risk of stroke was more significant in women than in men. However, P for interaction between the sex are not reported.

Response: We apologize for this confusion. We did not want to emphasize the difference of stroke risk between women and men. We have revised our statement in the manuscript:

“Results of sex-stratified analyses are shown in Table 4. In original models, higher levels of FBG were significantly associated with an increased risk of total stroke only in women. After adjustment covariate for age, BMI, and other variates, the risk association remained significant (HR: 1.92, 95%CI: 1.22-3.01, P=0.004) for the fourth level in female groups.” (Please see page 11, lines 225-229)

Discussion:

13. I think it is interesting that even in patients with prediabetes (IFG) defined according to the WHO or ADA criteria, the risk of stroke was both increased. These results support the notion that prediabetes is not only a high risk of progression into diabetes, but also a risk factor for cardiovascular morbidity. Based on this notion, the author should refer these important studies: PMID: 32669282 and PMID: 27881363: these important meta-analyses had reported that prediabetes is a risk factor of CVD (including stroke) and all-cause mortality.

PMID: 33769672: this large sample meta-analysis reported prediabetes is associated with the risk of heart failure, another important comorbidity of dysregulation of blood glucose.

Response: Thanks for your suggestions. The revised statement is quoted below.

“Results based on the ADA and WHO criteria demonstrated that FBG levels of ≥7.0 mmol/L were a significant risk factor for stroke [HR (95% CI): 1.68 (1.26, 2.55) and 1.60 (1.21, 2.12) following the

ADA and WHO criteria, respectively], whereas the risk of stroke in patients with IFG had an increase trend but this trend was not statistically significant." (Please see page 12, lines 271-275)
"Although diabetes is associated with stroke, the influence of prediabetes on future stroke risk has not been clear yet. Some large sample meta-analyses reported prediabetes is associated with the risk of CVD[28 29] and heart failure[30]. But in another meta-analysis including 15 cohort studies, more than half of the studies showed that after adjusting for cardiovascular risk factors, there was no significant association between pre-diabetes and stroke[31]. A cohort study investigated the sex-specific associations of pre-diabetes with major clinical outcomes reached the same conclusion[32]." (Please see page 13, lines 280-287)

14. Limitation: some important risk factors for stroke were not measured and adjusted, e.g, LDLC, eGFR.
Response: Thanks for your suggestions. We have available data of LDL-C and eGFR in the study and therefore we have further adjusted for LDL-C, eGFR, together with the use of statins in the revised manuscriptlly (please see Table 2-4 and Supplemental appendix S2)

VERSION 2 – REVIEW

REVIEWER	Huang, Yuli Shunde Hospital of Southern Medical University, Cardiology
REVIEW RETURNED	14-Jul-2021

GENERAL COMMENTS	I think the manuscript is well revised and had no further comment.