Peer Review File

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Reviewer A

The authors investigated the association between triglyceride levels and cardiovascular disease (CVD) as well as all-cause mortality in younger Chinese adults, age < 40 years old (18-39 years old) longitudinally.

Overall comments:

Comment 1: Address an important issue concerning TG levels and their association with cardiovascular disease (vascular disease) in Chinese adults 18 < age < 40 years old at the onset of the study, since many studies have been performed on older adults.

Reply 1: Thank you for appreciating our work and offering constructive comments. The responses to your valuable comments are provided below:

Comment 2: The management of low-density lipoprotein cholesterol (LDL-C) in preventing CVD is already established, but how well TG levels should be managed is still unclear, especially starting at a younger age. However, in order to fully assess the relationship between TG levels and the risk of CVD development, there are many factors which should be considered (see below), ruled out or at least mentioned such as various causes of hypertriglyceridemia (HTG), secondary causes of HTG, various factors which increase the risk of CVD development as well as biological mechanisms. HTG is an extremely heterogeneous conditions and there are many causes of TG elevations.

Reply 2: We thank you for this valuable comment. We agree with the opinion that many factors should be considered, ruled out, or at least mentioned such as various causes of hypertriglyceridemia (HTG). We have addressed those factors accordingly as below.

Comment 3: ApoB levels would have been useful in deciphering which lipoprotein is the source of TG short of sorting for VLDL vs chylomicrons. Lipoprotein (a) is also an important lipid factor for consideration in the development of CVD.

Reply 3: We thank you for this valuable comment. We entirely agree with the opinion that "lipoprotein (a) is an important lipid factor in the development of CVD". It is a pity that we lack the information of lipoproteins.

Changes in the text: Therefore, we added a limitation in the revised manuscript "Second, although we have made every effort to adjust for some important confounders, there is always a chance of unmeasured residual confounding (such as level of lipoproteins (***JAMA Cardiol.*** *2022* Jul 1;7(7):760-769) and hypertriglyceridemia-prone medications use)." (See page 12, lines 18 to 20)

Comment 4: Diabetes mellitus (DM) and alcohol use are major contributing factors for HTG, and hypertension is a major risk for CVD development. Therefore, these should have been taken into consideration in the analysis as well as HTG-prone medications such as estrogen, etc.

Reply 4: We thank you for this valuable comment. We agree with the opinion that diabetes mellitus (DM) and alcohol use are major contributing factors for HTG, and hypertension is a major risk for CVD development.

Changes in the text: Therefore, we have adjusted for alcohol consumption, DM, and hypertension as confounders in model 2 (See page 7, lines 17 to 18) and added related sensitivity analyses as advised (See **Supplementary Tables 3 to 4**).

In addition, as we lack the information on HTG-prone medications use, we have addressed this limitation in the revised manuscript "Second, although we have made every effort to adjust for some important confounders, there is always a chance of unmeasured residual confounding (such as level of lipoproteins(***JAMA Cardiol.*** *2022* Jul 1;7(7):760-769) and hypertriglyceridemia-prone medications use)." (See page 12, lines 18 to 20)

<u>Specific comments:</u>

Abstract:

Comment 5: Recommend editing the abstract after the main text has been edited.

Reply 5: We thank you for this kindly comment. We have edited the abstract according to the revised main text as advised. (please see the red font in the revised abstract)

Introduction:

Comment 6: In this section, it is important and should not forget to mention about the well-known and well-documented direct relationship between low-density lipoprotein cholesterol (LDL-C) levels and CVD since this has been well established though the studies of patients with familial hypercholesterolemia (FH), and these patients typically do not have high triglyceride (TG) levels. In addition, lipoprotein (a) elevation is also a risk factor of CVD (Duarte Lau F, Giugliano RP. Lipoprotein(a) and its Significance in Cardiovascular Disease: A Review. JAMA Cardiol. 2022;7(7):760–769. doi:10.1001/jamacardio.2022.0987). All the guidelines suggest managing LDL-C first, and then, considering TG level management. Therefore, the authors should include about LDL-C levels before mentioning TG levels and the risk of CVD.

Reply 6: We thank you for this valuable comment. We agree with the opinion of "managing LDL-C first, and then, considering TG level management". We have modified our introduction as advised. As the paper is not focused on lipoprotein (a) and we lack information on lipoprotein, we have added a limitation in the revised manuscript.

Changes in the text: We have added some information related to "LDL-C" in the Introduction section "Low-density lipoprotein cholesterol (LDL-C) is a well-established risk factor for CVD. In recent years, growing evidence suggests that elevated triglyceride (TG) levels may contribute to residual cardiovascular risk, even in patients with controlled LDL-C levels (***Pharmacol Ther***. 2022 Sep: 237:108172)". (See page 4, lines 8 to 10)

In addition, we have added a limitation in the revised manuscript "Second, although we have made every effort to adjust for some important confounders, there is always a chance of unmeasured residual confounding (such as level of lipoproteins (***JAMA Cardiol.*** *20*22 Jul 1;7(7):760-769) and hypertriglyceridemia-prone medications use)." (See page 12, lines 18 to 20)

Comment 7: Technically speaking stroke is cerebral vascular disease, not necessarily cardiovascular disease (CVD). Maybe change CVD to "vascular disease"? Also, please consider the biological mechanisms" of MI, ischemic and hemorrhagic stroke. MI is typically considered an ischemic event and when mentioning about plaque formation (atherosclerosis), it is less likely to lead to hemorrhagic events. https://www.ncbi.nlm.nih.gov/books/NBK559173/#:~:text=type%20of%20stroke.-,Hemorrhagic

%20stroke%20is%20due%20to%20bleeding%20into%20the%20brain%20by,bleeding%20into%20the%20subarachnoid%20space. Cerebrovascular accident (CVA), otherwise called a stroke, is the third major cause of morbidity and mortality in many developed countries. Stroke can be either ischemic or hemorrhagic. Ischemic stroke is due to the loss of blood supply to an area of the brain. It is a common type of stroke. Hemorrhagic stroke is due to bleeding into the brain by the rupture of a blood vessel. Hemorrhagic stroke may be further subdivided into intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH). ICH is bleeding into the brain parenchyma, and SAH is bleeding into the subarachnoid space. Hemorrhagic stroke is associated with severe morbidity and high mortality. Progression of hemorrhagic stroke is associated with worse outcomes. Early diagnosis and treatment are essential given the usual rapid expansion of hemorrhage, causing sudden deterioration of consciousness and neurological dysfunction.

Reply 7: We thank you for raising this important point. Although we have defined cardiovascular disease (CVD) according to previous studies with high quality (***J Am Coll Cardiol***. 2020 Jun 16;75(23):2921-2930; ***JAMA Neurol.*** 2021 Nov 1;78(11):1367-1374; ***EClinicalMedicine***. 2022 Dec 2:55:101761. ***Environ Int***. 2019 Apr: 125:51-57), we agree with the reviewer's opinion that hemorrhagic stroke is different from the other two in term of etiology. We think it is more suitable to eliminate hemorrhagic stroke from the CVD composite outcome. The modified results with a CVD event composed only of myocardial infarction and ischemic stroke were presented in the revised manuscript. (See page 6, line 17)

Comment 8: Also, it would be important to briefly mention about causes of increase TG levels, and secondary causes are important factors which should be considered in patients in the study. Lars Berglund, John D. Brunzell, Anne C. Goldberg, Ira J. Goldberg, Frank Sacks, Mohammad Hassan Murad, Anton F. H. Stalenhoef, Evaluation and Treatment of Hypertriglyceridemia: An Endocrine Society Clinical Practice Guideline, The Journal of Clinical Endocrinology & Metabolism, Volume 97, Issue 9, 1 September 2012, Pages 2969–2989, https://doi.org/10.1210/jc.2011-3213.

Reply 8: We thank you for this valuable comment. We agree with the opinion that "it is important to briefly mention about causes of increased TG levels, and secondary causes are important factors which should be considered in patients in the study".

Changes in the text: We modified the text as "To assess the robustness of our results, we also performed the following sensitivity analyses excluding participants with factors that impact TG levels, CVD events, or all-cause mortality (***J Clin Endocrinol Metab***. 2012 Sep;97(9):2969-89; ***Hypertension***. 2020 Feb;75(2):285-292)". (See page 8, lines 3 to 4)

In addition, we have added several sensitivity analyses in the revised manuscript. (See **Supplementary Tables 3 to 4**)

Comment 9: In addition, majority of TG molecules are carried in two different lipoproteins, chylomicrons, and very-low density lipoproteins (VLDL), and the type lipoproteins is an important factor. It is well-known that patients with familial chylomicronemia syndrome (FCS) have a low risk of CVD despite of extremely high TG levels (> 1,000 mg/dL) since birth. Therefore, it is important to distinguish between TG carried in different lipoproteins. Pancreatitis is the major risk for patients with FCS. Chylomicronemia due to multi-factorial causes have more risk of CVD, but multi-factorial hypertriglyceridemia (HTG) which is the most common type would have a high level of apoB, and higher risk of CVD. Thus, it is important to recognize the heterogeneity of HTG. Martine Paquette, Sophie Bernard, Robert A. Hegele, Alexis Baass, Chylomicronemia: Differences between familial chylomicronemia syndrome and multifactorial chylomicronemia, Atherosclerosis, Volume 283, 2019, Pages 137-142, ISSN 0021-9150, https://doi.org/10.1016/j.atherosclerosis.2018.12.019.

Reply 9: We thank you for this valuable comment. We entirely agree with the opinion that "the majority of TG molecules are carried in two different lipoproteins, chylomicrons, and very-low-density lipoproteins (VLDL), and the type lipoproteins is an important factor……". And we have modified the manuscript as advised.

Changes in the text: We have modified the text "Third, the majority of TG molecules are carried in two different lipoproteins: chylomicrons and very low-density lipoproteins. It is well-known that patients with familial chylomicronemia syndrome (FCS) have a low risk of CVD despite extremely high TG levels (> 1,000 mg/dL) since birth (***Atherosclerosis***. 2019 Apr: 283:137-142). Therefore, the type of lipoproteins, rather than TG itself, is an important factor." (See page 11, lines 4 to 8)

Comment 10: TG in VLDL or apolipoprotein B100 containing lipoproteins are the ones which are known to be associated with an increased risk of CVD. Therefore, it is more informative to have the information on apoB levels. Chylomicrons carry apoB48 which is a truncated version of apoB100 by RNA editing in the intestine. Sniderman AD, Thanassoulis G, Glavinovic T, et al. Apolipoprotein B Particles and Cardiovascular Disease: A Narrative Review. JAMA Cardiol. 2019;4(12):1287–1295. doi:10.1001/jamacardio.2019.3780. In recent years, TG has gained more interest in its relationship to CVD, but it is important that the overall lipoprotein metabolism should be understood and briefly discussed in the introduction.

Reply 10: We thank you for this valuable comment. We agree with the opinion that "It is more informative to have the information on apoB levels". As we lack the information of apolipoproteins, we have not mentioned the information in the Introduction section.

Comment 11: Also, there are some well-established modifiable risk factors which have been published some of which have been mentioned in this review, and these should be considered and excluded so that the relationship between TG levels and CVD risk is clearer. Hajar R. Risk Factors for Coronary Artery Disease: Historical Perspectives. Heart Views. 2017 Jul-Sep;18(3):109-114. doi: 10.4103/HEARTVIEWS.HEARTVIEWS_106_17. PMID: 29184622; PMCID: PMC5686931.

Reply 11: Thank you for this valuable comment. We agree with the opinion "there are some well-established modifiable risk factors which should be considered and excluded so that the relationship between TG levels and CVD risk is clearer".

Changes in the text: Therefore, we have adjusted for alcohol consumption, current smokers, DM, and hypertension as confounders in model 2 (See page 7, lines 17 to 18) and added related sensitivity analyses as advised (See **Supplementary Tables 3 to 4**).

Comment 12: Lines 7: "Contributor", this is true, but it has a more direct relationship so "cause" may be a better choice of word though this is a very subtle point. Smoking is a "contributor". Also, consider changing "disability" to "morbidity". This is also a very minor comment.

Reply 12: Thank you for noticing these errors. We have changed these words in the revised manuscript as advised.

Changes in the text: We have rewritten the term "contributor to disability" to "cause of morbidity" in the revised manuscript. (See page 4, line 2)

Comment 13: Line 9: "Lifestyle changes and/or medications" suggest changing a singular to plural. Or "a lifestyle change".

Reply 13: Thank you for this valuable comment. We have rewritten the terms in the revised manuscript as suggested.

Changes in the text: We have rewritten the terms "lifestyle change and/or medication" to "lifestyle changes and/or medications" in the revised manuscript. (See page 4, Line 4)

Comment 14: Line 10-11: Consider editing the sentence. "… to prevent CVD by slowing the development and progression of plaque …".

Reply 14: Thank you for this valuable comment. We have rewritten the sentence as suggested.

Changes in the text: We have rewritten the sentence "…to prevent CVD by slowing the progression of atherosclerotic plaque …" to "…to prevent CVD by slowing the development and progression of plaque …". (See page 4, line 5)

Comment 15: Lines 12-16: Please consider editing the paragraph to include the known facts about the relationships between lipoproteins and CVD and TG.

Reply 15: Thank you for this valuable comment. We think it is more suitable to discuss the relationships between lipoproteins and CVD and TG in the discussion section.

Changes in the text: We have modified the text "Third, the majority of TG molecules are carried in two different lipoproteins: chylomicrons and very low-density lipoproteins. It is well-known that patients with familial chylomicronemia syndrome (FCS) have a low risk of CVD despite extremely high TG levels (> 1,000 mg/dL) since birth (***Atherosclerosis***. 2019 Apr: 283:137-142). Therefore, the type of lipoproteins, rather than TG itself, is an important factor." (See page 11, lines 4 to 8)

Comment 16: Line 19: Do the authors mean "among adults between 40-75 years old. Not "young"

Reply 16: Thank you for noticing this error. We have deleted the word "young" in the revised manuscript as you advised.

Changes in the text: We have deleted the word "young" in the term "young adults" as advised. (See page 4, line 18)

Methods:

Comment 17: Line 31: What is the lower limit of the age for the study? Who are considered adults age > 18 or > 20 or > 21 years old? Or does it mean that children or adolescents were also included in the study? Later it is mentioned that 19-39 years old in the discussion section, but should have been mentioned here.

Reply 17: Thank you for noticing this error. Participants in our study were adults aged>18 and <40 years old. Since the term "adults" refers to males or females aged>18 years, we have made uniform changes in the revised manuscript.

Changes in the text: We have rewritten the related information about participants' age as "adults younger than 40 years" in the revised manuscript. (See page 3, line 4; page 4, line 20; page 10, line 7; page 12, line 13)

Comment 18: It is important that secondary factors which are known to increase TG should have been considered for exclusion. Alcohol can raise TG levels as well as certain medications. Hypertension is a well-known risk factor for CVD. Fuchs FD, Whelton PK. High Blood Pressure and Cardiovascular Disease. Hypertension. 2020 Feb;75(2):285-292. doi: 10.1161/HYPERTENSIONAHA.119.14240. Epub 2019 Dec 23. PMID: 31865786; PMCID: PMC10243231.

Reply 18: Thank you for this valuable comment. We agree with the opinion that "It is important that secondary factors which are known to increase TG should have been considered for exclusion".

Changes in the text: Therefore, we have adjusted for alcohol consumption, current smokers, DM, and hypertension as confounders in model 2 (See page 7, lines 17 to 18) and added related sensitivity analyses as advised (See **Supplementary Tables 3 to 4**).

Comment 19: Line ~7: Diabetes diagnosis typically requires multiple readings of fasting glucose and ideally also to have HgbA1C levels along with fasting glucose. "Self-reported history" may not be an ideal criterion. What are the diagnostic criteria used in China?

Reply 19: Thank you for this valuable comment. The diabetes diagnostic criteria used in China are close to the American Diabetes Association criteria (***Diabetes Care***.2021 Jan; 44(Suppl 1): S15-S33). The diagnostic criteria of diabetes in our paper are according to previous studies with high quality (***Diabetes Care***. 2021 Jun 1;44(6):1426-1432; ***JAMA***. 2021 Dec 28;326(24):2498-2506).
Changes in the text: To describe the diagnostic criteria more clearly, we have modified the text "Diabetes was defined as self-reported physician-diagnosed type 2 diabetes……". (See page 6, lines 13 to 14)

Comment 20: In this genomic era, ideally patients with monogenic causes of HTG, especially FCS or patients with FH (less likely) are excluded from the study. It would be great to know whether patients with TG carry FCS-related gene variant (lipoprotein-lipase (LPL), apolipoprotein C-II (APOC2), apolipoprotein A-V (APOA5), high-density lipoprotein binding protein 1 (GP1HBP1), and lipase maturation factor 1 (LMF1)) in one allele among the study cohort, but this could be another study in the future.
Reply 20: Thank you for this valuable comment. We entirely agree with the opinion that "ideally patients with monogenic causes of HTG, especially FCS or patients with FH (less likely) are excluded from the study". It is a pity that we lack the information on genomics.

Results:
Comment 21: Line ~6: "current drinkers" should have been excluded due to secondary effect on raising TG levels. Diabetes mellitus (DM) is also a secondary cause of raising TG levels, and the control of DM becomes an important factor. DM itself is a factor to increase CVD without having high TG; therefore, to assess an unhindered effect of TG elevation on the risk of CVD, it may be important to exclude patients with DM from the study or patient to have a certain level of DM control such as HgbA1C < 7%. Otherwise, for certain patients with DM, CVD may be due to DM. Schmidt AM. Diabetes Mellitus and Cardiovascular Disease. Arterioscler Thromb Vasc Biol. 2019 Apr; 39(4):558-568. doi: 10.1161/ATVBAHA.119.310961. PMID: 30786741; PMCID: PMC6532416.
Reply 21: Thank you for this valuable comment. We agree with the opinion that "DM itself is a factor to increase CVD without having high TG; therefore, to assess an unhindered effect of TG elevation on the risk of CVD, it may be important to exclude patients with DM from the study".

Changes in the text: Therefore, we have adjusted for alcohol consumption, and DM as confounders in model 2 (See page 7, lines 17 to 18) and added related sensitivity analyses as advised (See **Supplementary Tables 3 to 4**).

Discussion:

Comment 22: At the stage of study planning, strict consideration of the mechanisms of HTG as well as CVD or CVA. These should have been introduced briefly in the introduction. In addition, as stated above, secondary causes should have been considered and excluded from the study or grouped separately in the study. These considerations should have been done prior to the study as well as being mentioned in the introduction clearly. Secondary causes of HTG such as DM and alcohol as well as other TG-related medications etc. including estrogen or contraceptive pills should have been excluded or treated separately. Furthermore, obesity can be a secondary cause as well.

Reply 22: Thank you for this valuable comment. We agree with the opinion the reviewer advised above.

Changes in the text: Therefore, we have adjusted for alcohol consumption, DM, and obesity as confounders in model 2 (See page 7, lines 17 to 18) and added related sensitivity analyses as advised (See **Supplementary Tables 3 to 4**).

Comment 23: Smoking itself can be a risk for CVD. https://www.cdc.gov/tobacco/sgr/50th-anniversary/pdfs/fs_smoking_cvd_508.pdf How many cigarettes and length of time would have had effects on the vascular system as well.

Reply 23: Thank you for this valuable comment. We agree with the opinion that "Smoking itself can be a risk for CVD. How many cigarettes and length of time would have had effects on the vascular system as well". In the present study, current smokers were defined as those who smoked more than 1 cigarette per day on average over the past year, and this definition has been adopted by many studies (***JAMA Netw Open***. 2022 Jun 1;5(6): e2218323; ***Age Ageing***.2022 Jun 1;51(6): afac109). However, it is a pity that we lack more detailed information on the amount and duration of smoking.

Comment 24: Please re-consider using hemorrhagic stroke as one of the end-points. Ischemic is associated with plaque formation, but hemorrhagic is not (not typically related to atherosclerotic event).

Reply 24: We thank you for raising this important point. Although we have defined cardiovascular disease (CVD) according to previous studies with high quality (***J Am Coll Cardiol***. 2020 Jun 16;75(23):2921-2930; ***JAMA Neurol.*** 2021 Nov 1;78(11):1367-1374; ***EClinicalMedicine***. 2022 Dec 2:55:101761. ***Environ Int***. 2019 Apr: 125:51-57), we agree with the reviewer's opinion that "Ischemic is associated with plaque formation, but hemorrhagic is not". We think it is more suitable to eliminate hemorrhagic stroke from the CVD composite outcome. The modified results with a CVD event composed of only myocardial infarction and ischemic stroke were presented in the revised manuscript. (See page 6, lines 13 to 14)

Comment 25: Recommend to edit the paragraph starting, "The underlying mechanism between TG and the risk of CVD are unclear…" One needs to separate TG in chylomicrons vs TG in VLDL particles since high TG in chylomicrons are less likely to be associated with CVD. The risk is for pancreatitis. It may be important to mention about FCS as well.

Reply 25: Thank you for this valuable comment. We have modified the text as advised.

Changes in the text: We have modified the text "Third, the majority of TG molecules are carried in two different lipoproteins: chylomicrons and very low-density lipoproteins. It is well-known that patients with familial chylomicronemia syndrome (FCS) have a low risk of CVD despite extremely high TG levels (> 1,000 mg/dL) since birth (***Atherosclerosis***. 2019 Apr: 283:137-142). Therefore, the type of lipoproteins, rather than TG itself, is an important factor." (See page 11, lines 4 to 8)

Comment 26: Also, separating TG elevation due to secondary causes such as DM, metabolic syndrome, obesity etc. would be important in order to draw a credible conclusion. Patients with these conditions or with metabolic derangements are already known to be at high risk of developing CVD, so their TG levels are known to be higher than otherwise. Therefore, in order to address the relationship between "TG levels" and the risk of CVD, these additional factors should be considered and excluded.

Reply 26: Thank you for this valuable comment. We agree with the opinion that "separating TG elevation due to secondary causes such as DM, metabolic syndrome, obesity etc. would be important in order to draw a credible conclusion". We have modified the manuscript as advised.
Changes in the text: Therefore, we have adjusted for alcohol consumption, current smokers, DM, hypertension, and obesity as confounders in model 2 (See page 7, lines 17 to 18) and added related sensitivity analyses (including metabolic syndrome) as advised (See **Supplementary Tables 3 to 4**).

Comment 27: Thank you very much for the opportunity for allowing me to review this manuscript.
Reply 27: Thank you very much for taking your valuable time to provide us with these constructive suggestions.

Reviewer B

Comment 28: I didn't see if there was adjustment of survival according to Tg and other diseases DM HTN SMOKING BMI cholesterol (T CH, LDL HDL) HB CREAT ALBUMIN and other clinical.
Reply 28: Thank you for this valuable comment. In the revised manuscript, we have adjusted for as many confounders as possible including age, sex, obesity, high-density lipid cholesterol, low-density lipid cholesterol, smoking status, alcohol consumption, physical activity, education level, hypertension, diabetes, and family history of CVD.

Reviewer C

Comment 29: In the present study the authors reported association of high baseline TG levels with increased long-term CVD and all-cause mortality in Chinese young adults. The study is well designed and conducted and data very clearly presented and discussed.
Reply 29: Thank you for appreciating our work and offering constructive comments. The responses to your valuable comments are provided below:

There are some suggestions to improve the manuscript.

Comment 30: Why the authors performed COX regression only by quartiles and not also by using the whole range of TG levels?

Reply 30: Thank you for this valuable comment.

Changes in the text: We have performed COX regression for each primary outcome by using the whole range of TG levels as advised and the related results are presented in **Table 2**, **Table 4**, and **Supplementary Tables 3 to 4**.

Comment 31: The authors should perform and present Kaplan-Meier survival curves comparing the quartiles TGs.

Reply 31: Thank you for this valuable comment. We have performed and presented Kaplan-Meier survival curves comparing the quartiles TGs in **Figures 1 to 2**.

Comment 32: The authors should also perform ROC curve analyses to test discriminatory/predictive capacity of TGs for all-cause mortality as well as long-term composite CVD, and separately for MI and ischemic stroke.

Reply 32: Thank you for this valuable comment. ROC is usually used to evaluate the prediction efficiency of risk factors on outcome events. The current study is mainly aimed at exploring the association between TG and CVD events and all-cause mortality, which is a correlation study. Therefore, we have used the ROC to identify the optimal cutoff value (the best Youden Index: sensitivity + specificity −1) for each primary outcome. Then, we calculated the risk of participants with TG levels ≥ the optimal cutoff value compared with TG levels < the optimal cutoff value.