PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Relationship between triglyceride glucose index and the incidence of nonalcoholic fatty liver disease in the elderly: a retrospective cohort study in China
AUTHORS	Chen, Huanan; Li, Sangsang; Amoah, Adwoa Nyantakyiwaa; Bo, Yacong; Chen, Xuejiao; Shi, Zhan; Wan, Guodong; Huang, Jian; shi, songhe; Lyu, Quanjun

VERSION 1 - REVIEW

REVIEWER	Giovanni Tarantino Federico II University if Naples, Italy
REVIEW RETURNED	12-Jun-2020

GENERAL COMMENTS	I applaud the efforts made by authors dealing with a potentially interesting topic, even though plenty of other diagnostic proposals. I have some criticism: Triglycerides levels are strictly dependent from diet, exercise and lowering drugs. Thus, their concentrations are highly variable to be used as a fix parameter. Glicemia has the same issues, mainly if determined in T2DM pts. Consequently, if two parameters are not completely reliable, obviously their ratio has similar problems . With the reported sensitivity: 0.48, and specificity: 0.67, it is difficult to think of establishing this test as marker of NAFLD presence in any epidemiological study ....as authors state.....TyG index may be a novel predictor for NAFLD. Still, regular examination and evaluation of the TyG index might be useful for controlling the occurrence of NAFLD. Studying such a large population, a validation group could have been a good choice.

REVIEWER	Masahide Hamaguchi Japan
REVIEW RETURNED	30-Jun-2020

GENERAL COMMENTS	Chen Huanan et al. performed a retrospective study to investigate the impact of TyG index to predict an incident NAFLD in elderly. 1. Ref. 9 Dr. Fedchuk et al and Ref. 13 Dr. Kitae et al had already reported that TyG index could predict incident NAFLD. Authors should indicate the advantage of the current study comparing to the previous reports.

	2. Figures are low resolution. 3. Authors diagnosed 12765 subjects as NAFLD. I think these subjects should be called as subjects with fatty liver, not NAFLD, because a part of them intake alcohol more than 30g/day, or HBsAg positive or HCV Ab positive. 4. In Mediation analysis, authors selected age, sex, living alone, current smoking, exercise, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol as potential confounders. Authors should explain how they select them as potential confounders. 5. Authors think sex as a potential confounder. Therefore they should ROC analysis of TyG index as a predictor for incident NAFLD after separating subjects into men and women. 6. Please indicate ID approved by ethics committee.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

I applaud the efforts made by authors dealing with a potentially interesting topic, even though plenty of other diagnostic proposals.
Response: Thanks for your comments.

Question 1: Triglycerides levels are strictly dependent from diet, exercise and lowering drugs. Thus, their concentrations are highly variable to be used as a fix parameter. Glicemia has the same issues, mainly if determined in T2DM pts. Consequently, if two parameters are not completely reliable, obviously their ratio has similar problems.
Response 1:
1. Insulin resistance (IR) is considered a pivotal risk factor for NAFLD, and triglyceride-glucose (triglycerides, TyG) index was a reliable marker for IR. TyG index which was calculated with fasting triglycerides and fasting glucose. Previous studies had found higher TyG index significantly increased the risk of metabolic related diseases, such as coronary artery calcification, acute ST-elevation myocardial infarction (STEMI), T2DM, symptomatic coronary artery disease(1-4). What's more, our previous study also found that TyG index is an independent risk factor for incident CVD(5). The incidence of NAFLD was closely associated with the disorder of glucose and lipid metabolism as well as insulin resistance. Thus, we hypotheses that TyG index might be associated with the incident NAFLD.
2. TyG index was easily affected by lowering drug, thus we perform sensitivity analysis by excluded subjects with metabolic diseases such as T2MD, cardiovascular disease and stroke, which may take drugs that affect fasting triglycerides and fasting glucose, to lessen these effects. Sensitivity analysis found that the relationship between TyG and incidence of NAFLD was still stable (this result was added into the Supplementary materials: Supplementary Table 1).
3. Moreover, triglycerides and glucose were also affected by exercise and diet, on the one hand, TyG index was calculated by fasting triglycerides and fasting glucose, which somewhat reduced the effects of diet on TyG index. On the other hand, people's diet and exercise habits are closely related, that mean, people who exercise regularly tend to have healthier diet, regrettably, we missing the data of diet for subjects. While, after adjusted exercise, the HR of TyG index on NAFLD remained stable. This result indicated that diet was unlikely to change our conclusion.

Question 2: With the reported sensitivity: 0.48, and specificity: 0.67, it is difficult to think of establishing this test as marker of NAFLD presence in any epidemiological study ....as authors

state.....TyG index may be a novel predictor for NAFLD. Still, regular examination and evaluation of the TyG index might be useful for controlling the occurrence of NAFLD.
Response 2:
1. AUC is proverbially used as a method to assess the value of diagnosis and predictive discrimination, for it could provide the best cutoff value, sensitivity and specificity. While, AUC may not be an optimal method to predict future risk, because AUC only reflects one aspect of the model's predictive power. Although we observed relatively low sensitivity (0.48) and specificity (0.67) for baseline TyG index to diagnosis NAFLD: in our study, we didn't use TyG index as an indicator for the diagnosis of NAFLD, instead, we used it to predict the incident NAFLD. So, this result didn't affect that TyG index is an independent risk factor for incident NAFD.
2. In our research, we used restricted cubic spline analysis to further evaluate the association between TyG index and NAFLD, this result suggested that TyG index was nonlinear associated with incident NAFLD, and the reference value was determined according to the optimal cutoff value of the ROC curve.

Question 3: Studying such a large population, a validation group could have been a good choice.
Response 3: For our research, we applied different models to verify the effect of the TyG index on incident NAFLD. We will adopt your suggestions in the future study.

Reviewer 2:
Thank you very much for your comments and suggestions. We responded to your comments one by one.

Question 1: Ref. 9 Dr. Fedchuk et al and Ref. 13 Dr. Kitae et al had already reported that TyG index could predict incident NAFLD. Authors should indicate the advantage of the current study comparing to the previous reports.
Response 1:
Firstly, Ref. 9 was used TyG index to diagnose NAFLD, while the impact of the baseline level of TyG index on incident NAFLD did not involve in their research.
Secondly, Ref. 13 found that TyG index is significantly associated with incident NAFLD in Japanese which was consistent with our findings. What's more, compared with Dr. Kitae et al found, we further analyzed the effects of TyG index on incident NAFLD by restricted cubic spline analysis and mediation effects. We found that the relationship between TyG index and risk of NAFLD was nonlinear, and TyG index played a partial mediating role in the relationship between WHtR and NAFLD.

Question 2: Figures are low resolution
Response 2：We had resubmitted higher resolution figures.

Question 3: Authors diagnosed 12765 subjects as NAFLD. I think these subjects should be called as subjects with fatty liver, not NAFLD, because a part of them intake alcohol more than 30g/day, or HBsAg positive or HCV Ab positive.
Response 3: Ultrasound examination indicates the presence of steatohepatitis and after excluding the steatohepatitis caused by alcohol, viruses and drugs was defined as NAFLD, which was descripted in "1.3 NAFLD definition".

Question 4: In Mediation analysis, authors selected age, sex, living alone, current smoking, exercise, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol as potential confounders. Authors should explain how they select them as potential confounders.

Response 4: In mediation analysis, we selected age, sex, living alone, current smoking, exercise, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol as potential confounders based on the previous studies(5-11).

Question 5: Authors think sex as a potential confounder. Therefore, they should ROC analysis of TyG index as a predictor for incident NAFLD after separating subjects into men and women.
Response 5: we had performance the ROC analysis of TyG index to predictor for incident NAFLD by separating subjects into men and women. These results were showed in Supplementary materials: Supplementary Figure 1 and Figure 2, while the AUCs for men (0.587(95%CI:0.572-0.600)) and women (0.584(0.573-0.594)) were similar. What's more, we also performed restricted cubic spline analysis both for men and women, the relationship between TyG index and NAFLD had similar trends among men and women (Supplementary materials: Supplementary figure 3 and figure 4).

Question 6: Please indicate ID approved by ethics committee.
Response 6: The ID of ethics committee was showed in Ethics approval and the approve ID was ZZURIB202004, what's more, we added the approve ID to "1.1 subjects".

VERSION 2 – REVIEW

REVIEWER	Giovanni Tarantino Federico II University of Naples Italy Naples italy
REVIEW RETURNED	31-Jul-2020

GENERAL COMMENTS	I would appreciate it whether authors might deepen the following aspects. In fact, although some points are clarified, some others remain. IR was set to the best cut-off of 4.68 mg/dL, with a sensitivity of 96.5% and specificity of 85.0%, thus apparently reliable datum, in previous work, ie., J Clin Endocrinol Metab. 2010;95: 3347–3351. Considering that IR is the most important determinant, I dare say quite an exclusive mechanism of NAFLD, as even authors repeatedly state, how do authors can explain that the cut-off of their study is much higher, i.e., 8.63? Still, how do authors compare their results with a recent paper in which the cut-off is dramatically lower in a more severe form of NAFLD, i.e., NASH... i.e., Simental-Mendía LE, Simental-Mendía E, Rodríguez-Hernández H, et al. The product of triglycerides and glucose as biomarker for screening simple steatosis and NASH in asymptomatic women. Ann Hepatol. 2016;15: 715-720? Also, at the light that was used histology and not the US as a method in the aforementioned paper? Although something is reported, most of the answers' content given to this reviewer should be deeply emphasized as limitations to study. Subgroup analysis results should be put in the main text because it is a key aspect. Minor : line 44 1.019 (1.006,1.011) or 1.009?

REVIEWER	Masahide Hamaguchi Kyoto Prefectural University of Medicine, Japan
REVIEW RETURNED	28-Jul-2020

GENERAL COMMENTS	I think that this manuscript has a benefit to publish and authors answered all comments, which reviewers provided, and revised the manuscript well.

VERSION 2 – AUTHOR RESPONSE

Question 1: Please state any competing interests or state 'None declared':

Response to Question1: "All authors have no conflicts of interest" which is shown in the parts of Conflict of Interest (Page 13).

Question 2: I would appreciate it whether authors might deepen the following aspects. In fact, although some points are clarified, some others remain. IR was set to the best cut-off of 4.68 mg/dL, with a sensitivity of 96.5% and specificity of 85.0%, thus apparently reliable datum, in previous work, ie., J Clin Endocrinol Metab. 2010;95: 3347–3351.

Considering that IR is the most important determinant, I dare say quite an exclusive mechanism of NAFLD, as even authors repeatedly state, how do authors can explain that the cut-off of their study is much higher, i.e., 8.63?

Response to Question2: As you pointed, it is recognized that IR is closely associated with the incidence of NAFLD(1). And the best cut-off level of IR for was 4.68 mg/dL. While, in our research, we used TyG index, a reliable surrogate of IR, calculated by as ln [fasting triglyceride (mg/dl) ×fasting plasma glucose (mg/dl)/2], to study the relationship between the TyG index and the incidence of NAFLD. So, the best cut off value of TyG index was higher than that of IR.

Question 3: Still, how do authors compare their results with a recent paper in which the cut-off is dramatically lower in a more severe form of NAFLD, i.e., NASH... i.e., Simental-Mendía LE, Simental-Mendía E, Rodríguez-Hernández H, et al. The product of triglycerides and glucose as biomarker for screening simple steatosis and NASH in asymptomatic women. Ann Hepatol. 2016;15: 715-720?

Response to question 3: The cutoff value of TyG index was 4.58 in the study of Simental-Mendía LE et.al, which is lower than ours(8.63) (2). The different cutoff values may be caused by the following reasons.

Firstly, the participants of two studies were different. The participants of Simental-Mendía LE et.al were asymptomatic women aged 20 to 65 years in Mexico, while the participants of our study were elderly (60 years old or above) from China. A cohort study based on Chinese indicated that the best cutoff value for TyG index was 8.52(3), what's more, a cross-section study based on Chinese also found that the best cut off value of TyG for NAFLD was 8.5,which were generally similar to ours (4). Secondly, the study designs of two studies were different. Additionally, the methods to diagnosis NAFLD were different, they adopted liver biopsy to diagnosis NAFLD while we utilized ultrasonography to define NAFLD. Furthermore, the mean value of TyG index of us was higher (8.43 in participants without NAFLD in follow-up, and 8.61 in participants with NAFLD in follow up) than them (4.50 in normal liver while 4.95 in NASH). The above are the reasons that might cause the different cutoff values between two studies.

We also discussed this difference in our discussion “The results of ROC suggested that 8.63 was the best cutoff value of TyG index for predicting the incidence of NAFLD. while, the cut off value TyG index was higher than Simental-Mendía LE et.al(2),which might be caused by the following reasons. Firstly, the participants were different between two studies, in Simental-Mendía LE et.al research, the participants were from asymptomatic women aged 20 to 65 years in Mexico, while in our study, the participants were 60 or older from China (including men and women), moreover, the cutoff value in our research was similar to several studies from Asia(3-5); Secondly, study design of Simental-Mendía LE et.al is different from ours; What’s more, the methods to diagnosis NAFLD were different; Additionally, the mean values of TyG index were different. The above might be the reasons that resulted in different cutoff value.”

Question 4: Also, at the light that was used histology and not the US as a method in the aforementioned paper? Although something is reported, most of the answers' content given to this reviewer should be deeply emphasized as limitations to study.

Response to question 4:

We agree with the opinion of reviewer that liver biopsy is gold standard for diagnosis NAFLD, and gold standard is able to differentiate between simple steatosis and NASH. While it is unrealistic to primary screen NAFLD in general population. Ultrasonography is a less expensive than other advanced imaging methods, and is currently the most widely used imaging tool in clinic and the most acceptable method for the first-line screening of steatosis(6). Ultrasound could find high-risk groups of NAFLD, and then liver biopsy was performed on high-risk groups of NAFLD, which improves cost-effectiveness and saves social resources.

Furthermore, we discussed this limitation in our discussion“liver biopsy is gold standard to diagnosis NAFLD, while, in this study, we executed abdominal ultrasound to diagnosis NAFLD. While, liver biopsy is unrealistic to screen NAFLD in general population for the prevalence of NAFLD is high. Previous study also found that abdominal ultrasound is less expensive than other advanced imaging methods, and is currently the most widely used imaging tool in clinic and the most acceptable method for the first-line screening of steatosis”.

Question 5: Subgroup analysis results should be put in the main text because it is a key aspect.

Response to question 5: In our study, we found that the cut-off value of TyG for NAFLD was lower in men (8.68) than women (8.75), and the results of subgroup of ROC were added into main text (Figure 2B for men and Figure 2C for women, respectively). Sex subgroup of restricted cubic spline analysis was showed in Figure 3B for men and Figure 3C for women in main text.

References

1. KHAN R S, BRIL F, CUSI K, NEWSOME P N. Modulation of Insulin Resistance in Nonalcoholic Fatty Liver Disease. Hepatology 2019; 70(2): 711-24.

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3. ZHENG R, DU Z, WANG M, MAO Y, MAO W. A longitudinal epidemiological study on the triglyceride and glucose index and the incident nonalcoholic fatty liver disease. Lipids Health Dis 2018; 17(1): 262.

4. ZHANG S, DU T, ZHANG J, et al. The triglyceride and glucose index (TyG) is an effective biomarker to identify nonalcoholic fatty liver disease. Lipids Health Dis 2017; 16(1): 15.

5. KITAE A, HASHIMOTO Y, HAMAGUCHI M, OBORA A, KOJIMA T, FUKUI M. The Triglyceride and Glucose Index Is a Predictor of Incident Nonalcoholic Fatty Liver Disease: A Population-Based Cohort Study. Can J Gastroenterol Hepatol 2019; 2019: 5121574.

6. YU Y, CAI J, SHE Z, LI H. Insights into the Epidemiology, Pathogenesis, and Therapeutics of Nonalcoholic Fatty Liver Diseases. Adv Sci (Weinh) 2019; 6(4): 1801585.

VERSION 3 - REVIEW

REVIEWER	Tarantino Giovanni Italy
REVIEW RETURNED	10-Aug-2020

GENERAL COMMENTS	I see their point. A large discussion on mechanisms of NAFLD is redundant.

VERSION 3 – AUTHOR RESPONSE

Question1: Please state any competing interests or state ‘None declared’: I do not have a conflict of interests.

Response to Question1: I do not have a conflict of interests. What’s more, Conflict of Interest also declared that “All authors have no conflicts of interest”, as shown on Page 13 in main text.

Question 2: A large discussion on mechanisms of NAFLD is redundant.

Thanks for your suggestions again, we have made corresponding deletions to the mechanisms of NAFLD without affecting our meanings, and revised content of the discussion on the mechanism is “ The mechanism of IR on NAFLD could be explained by the following reasons. On one hand, IR has a direct effect on metabolism of glucose and lipid, and thus participates in the incidence and development of NAFLD[30]. Insulin resistance reduces glucose uptake in the adipose tissues and muscles, and reduces the hydrolysis of triglycerides in adipose tissue. Meanwhile, High insulin levels can increase the uptake of free fatty acids in the liver and the synthesis of TG, causing excessive accumulation of fat in the liver, which could initiate steatosis and then lead to the occurrence of NAFLD [7, 34, 35]. On the other hand, IR is always linked to chronic mild inflammation caused by the release of inflammatory factors, such as TNFα, IL-6, IL-1 and monocyte chemotactic protein-1, which can in turn promote IR and participate in the development and progression of NAFLD[36, 37].

Obesity is a key factor in development and progression of NAFLD. It has been documented that BMI is associated with incidence of NAFLD[38, 39]. While growing evidences suggested that the determinant of IR is not the degree of obesity, but the distribution of fat [40-43]. Central obesity can lead to inflammatory, oxidative stress and metabolic disorders, which are related to the development of IR[44, 45]. In this study, WHtR, an indicator of central obesity, was associated with NAFLD and the effect was mediated by TyG index among the study participants. This could be that central obesity leads to an increase in the TyG index and thus results in NAFLD.”