PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association between recovery/occurrence of metabolic syndrome and rapid estimated glomerular filtration rate decline in middle-aged and older populations: evidence from the China Health and Retirement Longitudinal Study
AUTHORS	Liu, Peijia; Tang, Leile; Fang, Jia; Chen, Chaojin; Liu, Xun

VERSION 1 – REVIEW

REVIEWER	Bragg-Gresham, Jennifer University of Michigan , Internal Medicine - Nephrology
REVIEW RETURNED	25-Jan-2022

GENERAL COMMENTS	This paper covers and important topic that has shown mixed results in the literature, the relationship between metabolic syndrome and kidney disease. While the results are plausible and add to the body of knowledge on this topic, the paper is a bit confusing and could benefit from editing. A detailed list is included below: Abstract: Needs more detail on methods: • State that MetS measurements were 4 years apart before the conclusion • State definition for rapid eGFR decline • Were only individuals without kidney disease included? – I see yes in the methods, but maybe state sooner. Introduction: • Was a standard/International definition of Metabolic Syndrome used? If not, in methods, can you say how it differs from other guidelines? Methods: • Many individuals were lost due to exclusion criteria or missing values (17,708 to 4,142). Do you know how these 4,142 compare to the full sample? • MetS severity score is interesting idea, but need to say clearly and sooner what cut-off you used…I'm still looking for that. Did you simply adjust using the continuous variables? • Assuming covariates were collected at baseline (i.e., important for eGFR). • MetS as confounding variable?? I thought these were the main predictor. Need to be clear if this is starting/baseline MetS, since change in MetS is the main predictor…wait, it is change in central obesity that is main predictor? This needs to be made more clear. Results:

	• Although fasting glucose and creatinine values were statistically different between fast decliners and non-fast decliners, they may not be clinically meaningful differences. • How can one explain that the slower decliners are the ones that start off with more risk factors…larger waist, etc. • Why is there no model with all 4 MetS change groups included? Looks like you only compared chronic to recovery and then developed vs. free. • For Table 3, are each of the components run in their own model (to predict rapid eGFR decline) or were these all in the same model? I'm sure many are collinear and that could explain why only central obesity is significant. Discussion: • Be careful using the word "risk" for "odds"…second sentence. Logistic models give you the odds, not the risk. • Consider that not enough time has passed for those who have developed MetS to show an association with eGFR. Needs more discussion of the clinical relevance of the findings.

REVIEWER	Chen, Jianghua First Affiliated Hospital, College of Medicine, Zhejiang University, Kidney Disease Center
REVIEW RETURNED	26-Jan-2022

GENERAL COMMENTS	1 Study lacks univariate analysis 2 Have confounding factors been adjusted for replicates? creatinine and eGFR classification, MetS scores and body mass index

REVIEWER	Chang, Che-Wei Kaohsiung Medical University Hospital
REVIEW RETURNED	18-Mar-2022

GENERAL COMMENTS	This is a longitudinal cohort study of the association between metabolic syndrome and eGFR decline based on a health database. The author first excluded individual whose baseline eGFR is < 60ml/min/1.73m2 and those who have clinician-reported malignant tumor, heart disease, stroke or kidney disease. However, some individuals may have renal stone disease, obstructive uropathy, autoimmune disease or even a acute urinary tract infection at the time of blood sampling. This could lead to selection bias. After 4 years of follow-up, the similar questions are raised. Those patients with declined eGFR may have acute urinary tract infection, obstructive uropathy and other autoimmune diseases. Thus, the Model 2 multivariate analysis should include confounding factors such as renal stone diseases, obstructive uropathy (BPH) and pyuria etc. 1. Are there any other blood tests, urine anlysis or self-reported diseases in your database that should be included in the analysis?

	In addition, the central obesity and elevated blood sugar should also be included in the confounding factors because there is significant difference between rapid eGFR decline group and non-rapid decline group. It may lead to errors in the multivariate regression model 2. If we included all the statistical significant variables ($p<0.05$) in the multivariate regression analysis, what are the OR ratio of each component of Mets? The author concluded that MetS recovery was associated with a reduced risk of rapid eGFR while MetS occurrence was not related to rapid eGFR decline in 4 years follow up. 3. Is it possible that those with metabolic syndrome might receive therapy or medication at the beggining of the health exam? Especially for those with hypertension, elevated fasting sugar and dyslipidemia, they could take medication during follow up. 4. Newly onset metabolic syndrome is actually associated with decline of renal function in these cohorts (https://pubmed.ncbi.nlm.nih.gov/34197633/) (https://pubmed.ncbi.nlm.nih.gov/31642000/) Authors should illustrate more about the relationship and time sequence between Mets and eGFR.

VERSION 1 – AUTHOR RESPONSE

Replies to Dr. Jennifer Bragg-Gresham (Reviewer #1)
Thank you for your professional comment. Your comments are constructive and helpful for us in order to revise and improve our paper. All suggested comments have been addressed as shown below.

Major Comments:
1. Abstract: Needs more detail on methods:
• State that MetS measurements were 4 years apart before the conclusion
• State definition for rapid eGFR decline
• Were only individuals without kidney disease included? – I see yes in the methods, but maybe state sooner.
Response: Thank you for your suggestion. According to your suggestions, we have stated that MetS were measured at both the beginning and the end of the 4-year follow-up. Besides, we have described more detail on methods including the rapid eGFR decline definition, exclusion criteria for participants and main statistical method. (Line 31-39)

Introduction:
2. Was a standard/International definition of Metabolic Syndrome used? If not, in methods, can you say how it differs from other guidelines?
Response: There are several diagnostic criteria for metabolic syndrome. Currently, there was no unified definition for MetS. The World Health Origination (WHO) diagnostic criteria proposed in 1999, the National Cholesterol Education Program Adult Panel Ⅲ (ATP Ⅲ) diagnostic criteria proposed in

2005, and International Diabetes Federation (IDF) diagnostic criteria proposed in 2006 were commonly used for metabolic syndrome. Other organizations like the American Association of Clinical Endocrinologist (AACE) and the European Group for the Study of Insulin Resistance (EGIR) used slightly different definitions but they were not as commonly used. All these diagnostic criteria mainly related to abdominal obesity, dyslipidemia, elevated blood glucose, and elevated blood pressure. However, these diagnostic criteria have different view of points and cut-off values for some specific indicators. For example, glucose metabolism dysfunction (insulin resistance or elevated blood glucose) was obligatory component for MetS diagnosis in 1999 WHO diagnostic criteria, while central obesity was obligatory component for MetS diagnosis in 2006 IDF diagnostic criteria. In this study, we adopted 2018 Chinese Guidelines for Prevention and Treatment of Hypertension (CGPTH) definition for metabolic syndrome diagnosis, which was similar to 2005 ATP Ⅲ. The difference lies mainly in the cutoff value of HDL and waist circumference. The waist cut point was 102 cm for men and 88cm for women, while HDL cholesterol cut point was 40 mg/dl for men and 50 mg/dl for women in 2005 ATP Ⅲ. According to 2018 CGPTH criteria, waist cut-off value was 90 cm in men and 85 cm in women, while HDL cut point was 40 mg/dL for both male and female. In methods, we briefly stated the similarities and differences of different MetS diagnostic criteria. (Line130-141)

Methods:

3.Many individuals were lost due to exclusion criteria or missing values (17,708 to 4,142). Do you know how these 4,142 compare to the full sample?

Response: Thank you for your professional comments. A large proportion of individuals were excluded due to missing values and exclusion criteria which would inevitably lead to bias. We have stated this limitation in the discussion (Line316-318). Meanwhile, we could directly obtain full sample characteristics such as age, gender, education level, marital status, height, weight, laboratory tests and other variables from the codebook published on the official CHARLS website. As shown in Figure 1, we only enrolled 7116 patients in 2011 at the beginning of the study. Hence, we compare the characteristics of the population included at the beginning and deleted during the follow-up (group 1, n = 2974) and the final included participants in the study (group 2, n =4142). There were no statistically significant differences in age, education level, drinking ratio, serum creatinine, fasting blood glucose, total cholesterol, triglyceride, hs-CRP, glycosylated hemoglobin, hemoglobin, height, abdominal circumference, hand grip strength, systolic blood pressure, diastolic blood pressure, mean arterial pressure, metabolic syndrome ratio between two groups (all $P > 0.05$, Supplemental table 1). Compared with group 2, group 1 had higher male ratio, proportion of smoking, weight, BMI, eGFR, and lower serum creatinine, high-density lipoprotein, serum uric acid, income, depressive symptom ratio, proportion of central obesity (all $P < 0.05$, Supplemental table 1). This has been added in the revised MS. Thank you again for your professional suggestion.

4. MetS severity score is interesting idea, but need to say clearly and sooner what cut-off you used…I'm still looking for that. Did you simply adjust using the continuous variables?

Response: Thank you for your professional suggestion. The objective of the study is to explore the association between MetS status and the rapid eGFR decline. However, MetS severity potentially affects the recovery/occurrence of MetS. For instance, individuals with high MetS severity may be less liable to recover. Similarly, for those without MetS, it is not straightforward to progress to severe MetS. Therefore, MetS scores were introduced to assess MetS severity in the study. Being an important covariate, the MetS severity score was found to have a linear relationship with logit conversion values of dependent variable (Rapid eGFR Decline) (Line 198-199, 201-203). Thus, as you said, we didn't convert it into a categorical variable and adjust using the continuous variable.

5. Assuming covariates were collected at baseline (i.e., important for eGFR).

Response: Thank you for your suggestion. We have added this important message in the Method of the revised MS (Line 172).

6. MetS as confounding variable?? I thought these were the main predictor. Need to be clear if this is starting/baseline MetS, since change in MetS is the main predictor…wait, it is change in central obesity that is main predictor? This needs to be made more clear.
Response: Sorry to confuse you in our earlier description. MetS is the core variable discussed in the study the MetS severity score was selected as confounding variables for model adjustments, but not the MetS (Line 204-206). Notably, central obesity is one of the diagnostic criteria for MetS, which included elevated blood glucose, elevated blood pressure, central obesity and dyslipidemia (Line 130-150). Change in MetS status was accompanied by changes of the diagnostic conditions including central obesity. Therefore, the analysis of central obesity is a subgroup analysis and part of sensitivity analysis. We have added the description of the part in method. (Line 208-212)

Results:
6.Although fasting glucose and creatinine values were statistically different between fast decliners and non-fast decliners, they may not be clinically meaningful differences.
Response: Thank you for your professional suggestion. I totally agreed with you. In this study, fasting blood glucose was used to determine the conditions for elevated blood glucose. Serum creatinine was used for eGFR calculation. Overall, although fasting glucose and creatinine values were statistically different between two groups, they may not be clinically meaningful differences. This has been added in the revised MS (Line 237-238).

7.How can one explain that the slower decliners are the ones that start off with more risk factors…larger waist, etc.
Response: Thank you for your professional comment. In the current study, we focus on the relationship between MetS changes and rapid eGFR decline during the four-year follow-up period. As a result, we think the indicator like larger waist at the baseline did not mean that the patients' MetS would develop or recover after 4-year follow-up period, and it could not describe the situation of patients at the end of the study. Alternatively, we focused on the impact of Mets syndrome or central obesity status changes on the rapid eGFR decline.

8. Why is there no model with all 4 MetS change groups included? Looks like you only compared chronic to recovery and then developed vs. free.
Response: Thank you for your professional comment. The purpose of this study was to observe the association between changes in MetS status and rapid eGFR decline, so we needed to maintain consistent MetS diagnosis at baseline status. Obviously, both MetS-free and MetS-developed groups did not have MetS at baseline, while both MetS-chronic and MetS-recovery groups had MetS at baseline. Thus, we only compared chronic to recovery and then developed vs. free in the study. This has been added in the limitation of our revised MS (Line 318-319).

9. For Table 3, are each of the components run in their own model (to predict rapid eGFR decline) or were these all in the same model? I'm sure many are collinear and that could explain why only central obesity is significant.
Response: Thank you for your professional comment. Each of the components runs in their own model to predict rapid eGFR decline in Table 3. According to your comment, we have added a description in the Notes of Table 3 (Line 447).

Discussion:
10.Be careful using the word "risk" for "odds"…second sentence. Logistic models give you the odds, not the risk.
Response: Thank you for your suggestion. We have corrected these descriptions.

11. Consider that not enough time has passed for those who have developed MetS to show an association with eGFR.

Needs more discussion of the clinical relevance of the findings.
Response: Thank you for your professional suggestion. One of the limitations of this study is the inability to know the exact time when MetS status change. As your suggestion states, for those in the MetS-devoloped group, the effects of MetS on renal function may be too short to observe a rapid decline in eGFR. Therefore, we need further follow-up in the future study. Besides, we have added more discussion in the revised MS according to your suggestion. (Line 272-277,282-284,287-291)

Replies to Prof. Jianghua Chen (Reviewer #2)
Response: Thank you for your professional comment. Your comments are constructive and helpful for us in order to revise and improve our paper. All suggested comments have been addressed as shown below.

1. Study lacks univariate analysis
Response: Thank you for your professional suggestion. We conducted univariate analysis in the supplemental table 2 to selected covariates for correction in the revised MS (Line 204-206; Line 237-238). Actually, Table 1 in the main text can also be used to screen calibration variables, and the results were almost consistent. Screening criteria for calibration variables were described in detail in the Method of main text. In general, there were three steps in the selection of calibration variables. Firstly, variables with $P < 0.15$ were retained; secondly, continuous variables that did not have a linear relationship with dependent variables were converted into categorical variables. Thirdly, we performed collinearity test between continuous variables and simplified the calibration variables. Finally, age, sex, serum creatinine, eGFR classification, grip strength classification; hemoglobin; MetS score and body mass index were selected for final model calibration.

2.Have confounding factors been adjusted for replicates? creatinine and eGFR classification, MetS scores and body mass index
Response: Thank you for your professional comment. Serum creatinine is the main variable for calculating eGFR. In general, eGFR and serum creatinine are collinear, and one of them is usually selected to calibrate the model in the logsitc model. However, during the linear relationship test, we found that the relationship of eGFR and the dependent variable were not linear, so we converted eGFR into a categorical variable. In this case, the simultaneous use of serum creatinine and eGFR grades for model calibration may not be significantly over-calibrated. As for MetS scores and body mass index, there was no obvious collinearity between two variables according the results of collinearity test. We have described the details in Statistical Methods (Line 198-206) and if you have any other confusion, we sincerely hope to explain for you later.

Replies to Dr. Che-Wei Chang (Reviewer #3)
1. Are there any other blood tests, urine anlysis or self-reported diseases in your database that should be included in the analysis?
In addition, the central obesity and elevated blood sugar should also be included in the confounding factors because there is significant difference between rapid eGFR decline group and non-rapid decline group. It may lead to errors in the multivariate regression model
Response: Thank you for your professional suggestion. We totally agreed with your suggestion. Renal stone disease, epiculopathy, epiculoepicardial disease or even acute urinary tract infection are related to the occurrence and development of renal disease. Unfortunately, there were no urine results or kidney ultrasound results in this prospective cohort, so some key information was inevitably missing. We have described these limitations in the discussion (Line 309-312). Secondly, most of the blood test results have been included in our table. Indicators such as white blood cell and hematocrit may be not related to the outcome of this study, so they are not included. Finally, there were other disease conditions such as memory impairment and asthma, but the incidence of these diseases were less than 5% of the overall population, so these diseases were not included. We included arthritis or rheumatism and its treatment, because the incidence of arthritis or rheumatism is more than 20% and

may affect renal function (Table 1). But these variables were not selected for model calibration. Besides, central obesity and elevated blood sugar are components of MetS. The problem of repeated calibration might occur if the variables are used for calibration, and these two variables are also discussed respectively in subgroup analysis.

2. If we included all the statistical significant variables ($p < 0.05$) in the multivariate regression analysis, what are the OR ratio of each component of Mets?
Response: Thank you for your professional Comment. According to the results of Table 1 in the main text and Supplemental Table 2, variables with P values < 0.15 were used to calibrate the logistic model. Continuous variables not presenting a linear relationship with the logit conversion value of the dependent variable were converted to categorical variables. Tolerance and variance inflation factors (VIFs) were used to test for collinearity. This existed if the tolerance was < 0.1 or the VIF was > 10. Eventually, age, sex, BMI, Scr, hemoglobin, eGFR classification, grip strength classification and MetS scores were selected as confounding variables for model adjustments in this study. If all variables with $P < 0.05$ are included in the calibration model, there may be the problem of repeated calibration.

The author concluded that MetS recovery was associated with a reduced risk of rapid eGFR while MetS occurrence was not related to rapid eGFR decline in 4 years follow up.
3. Is it possible that those with metabolic syndrome might receive therapy or medication at the beginning of the health exam? Especially for those with hypertension, elevated fasting sugar and dyslipidemia, they could take medication during follow up.
Response: Thank you for your professional comment. Interventions on blood glucose, lipids, and blood pressure can affect MetS status and renal outcomes, so we described the treatment of elevated blood pressure, elevated blood glucose, and dyslipidemia in our baseline data. However, these treatments were not statistically different between the rapid eGFR decline group and non-rapid eGFR decline group, so these treatments were not used for model calibration.

4. Newly onset metabolic syndrome is actually associated with decline of renal function in these cohorts (https://pubmed.ncbi.nlm.nih.gov/34197633/)
(https://pubmed.ncbi.nlm.nih.gov/31642000/)
Authors should illustrate more about the relationship and time sequence between Mets and eGFR
Response: Thank you for your professional suggestion. These two articles are also cited in this article. However, there are some differences between this study and the above-mentioned two articles in the definition of end points. In addition, the follow-up time of this study and those two studies are different. The present study found that the occurrence of Mets was not associated with a rapid decline in eGFR after a four-year follow-up. This does not mean that MetS has no effect on renal function, but it may be that the effect of MetS on renal function has not yet played a role, so further follow-up is required. These issues will also be further described in the discussion. (Line 272-277,282-284,287-291)

VERSION 2 – REVIEW

REVIEWER	Bragg-Gresham, Jennifer University of Michigan , Internal Medicine - Nephrology
REVIEW RETURNED	12-May-2022

GENERAL COMMENTS	Thank you to the authors for addressing the reviewer comments. I think the manuscript is strengthened, but still needs some attention. My individual thoughts/comments are below. The abstract seems confusing and out of order to me. The first sentence of the abstract refers to a knowledge gap that has not yet

	been defined. When you talk about the participants you mention the 4-year follow-up, which hasn't been introduced yet. I think this could be made clear by creating one methods section, unless the journal requires these headers in this order. I would find it much clearer to describe the sample along with the two time points in the methods. As it is now, we don't hear about the 4 groups of MetS until the results, which is not appropriate. Thank you for adding supplemental table 1. I think this is an important comparison. The patients did seem to be different on some characteristics. Can you discuss how you think this could affect your analyses? As for the final analyses, I do think you can analyze all four groups together as an additional (maybe supplemental) analysis. The four groups (independent variables) would be no MetS/no MetS, MetS/noMets, No MetS/MetS, and No MetS/No Mets. You would leave out the largest group as the reference.

VERSION 2 – AUTHOR RESPONSE

Replies to Dr. Jennifer Bragg-Gresham (Reviewer #1)
Thank you for your professional comment. Your comments are constructive and helpful for us in order to revise and improve our paper. All suggested comments have been addressed as shown below.

1.The abstract seems confusing and out of order to me. The first sentence of the abstract refers to a knowledge gap that has not yet been defined. When you talk about the participants you mention the 4-year follow-up, which hasn't been introduced yet. I think this could be made clear by creating one methods section, unless the journal requires these headers in this order. I would find it much clearer to describe the sample along with the two time points in the methods. As it is now, we don't hear about the 4 groups of MetS until the results, which is not appropriate.

Response: Thank you for your suggestion. We have re-write the Objectives section to make our objective more reasonable “Few studies have explored correlations between metabolic syndrome (MetS) alterations and renal deterioration in longitudinal cohorts. We aim to investigate associations between MetS recovery/development and rapid estimated glomerular filtration rate (eGFR) decline in the China Health and Retirement Longitudinal Study (CHARLS).” (Line 26-31).
Sorry for the confusion on the description of 4-year follow-up. CHARLS is a nationwide cohort of the middle-aged and elderly Chinese population, which was first investigated in 2011 and subsequently followed every 2-3 years. Blood draws were available in 2011 (Wave1) and 2015 (Wave3), so here we took Wave1 as baseline and Wave3 as cutoff time of follow-up for the study. We added this in the “Participants” and “Outcome measures” sections of revised Abstract (Line 36-39). “4142 participants with complete data were selected from the CHARLS during the 4-year follow-up period (2011-2015)”, “MetS were measured at 2011 and 2015 in CHARLS”.
Meanwhile, we have also added a further description in methodology of main text. “In total, 17,708 participants were registered at baseline (Wave 1 at 2011), of which 11,847 had blood sample tests” (Line 99-100). “no follow-up records and related blood examinations in Wave 3 at 2015.” (Line 104-105) “At baseline (Wave 1)” (Line 111). “Blood specimen testing in 2015 (Wave 3)” “Of note, the models and manufacturer information of blood test instruments in Wave 1 and Wave 3 were not available.” (Line 128-129)

2.Thank you for adding supplemental table 1. I think this is an important comparison. The patients did seem to be different on some characteristics. Can you discuss how you think this could affect your

analyses?

Response: Thank you for your suggestion.
As shown in Supplemental Table 1, group1 and group2 have differences in gender, serum creatinine, eGFR, smoking, household per capita income, marital status and other indicators, such as marital status and household per capita income, may not affect the rapid decline of eGFR or have an impact on the results of this study. However, gender is the final calibration variable of the Logistic model in this study, and the differences between group1 and group2 may bias the results. We have stated this in the Limitations section. “Fifthly, a large proportion of individuals were excluded due to exclusion criteria or missing values, and the basic characteristics between the 4142 enrolled participants and 2974 ones that excluded during follow-up might have biased some of our results”.

3.As for the final analyses, I do think you can analyze all four groups together as an additional (maybe supplemental) analysis. The four groups (independent variables) would be no MetS/no MetS, MetS/noMets, No MetS/MetS, and No MetS/No Mets. You would leave out the largest group as the reference.
Response: Thank you for your professional comment. The purpose of this study was to observe the association between changes in MetS status and rapid eGFR decline, so we needed to maintain consistent MetS diagnosis at baseline status. Obviously, both MetS-free and MetS-developed groups did not have MetS at baseline, while both MetS-chronic and MetS-recovery groups had MetS at baseline. In addition, there was no comparability between MetS-developed groups and MetS-recovery groups. Thus, we only compared chronic to recovery and then developed vs. free in the study.